European Eye Bank Association
XXII Annual Meeting
Sitges 22-23 January 2010

Organization

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With a special thank you to:

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Banco de Ojos, Centro de Oftalmología Barraquer

Dear Friends and Colleagues,

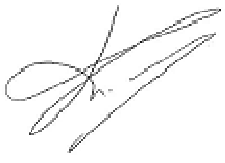
We would like to welcome you to the XXII Annual Meeting of the European Eye Bank Association. It is with great honor that we present this meeting. Our congress begins on January 22nd in the beautiful town Sitges, Spain. With the close proximity to Barcelona, Sitges offers a beautiful seafront setting for our meeting. Culture, History and Leisure are just steps away from our congress Hotel Meliá Sitges.

Our yearly association meetings always provide an excellent venue for sharing of ideas, scientific discussion, and networking within our ¨specialized¨ community. We hope to continue this tradition in this meeting. The European transplant community's ever-changing parameters for operation necessitate this ability to share in these intimate settings. During this particular session, we hope to add some focus to our discussions and scientific presentations toward:

¨**Donor selection: optimizing the inclusion/exclusion criteria**¨

Discussion regarding the impact of new regulations on eye banking activities will help clear a path to better management of donor selection and processing areas. Also, the technician sessions will provide a great opportunity for hands-on learning for eye bank personnel at all levels.

We are happy to welcome you.



Esteve Trias i Adroher, MD
Medical Director
Transplant Services Foundation

Juan P. Álvarez de Toledo Elizalde, MD
Head of Preservation Department
Banco de Ojos para el Tratamiento de la Ceguera

PROGRAM

Friday 22 January 2010

09:00 Technician Session at TSF
13:00 Board meeting
13:00 Registration

15:15 Opening Ceremony

Welcome of the President
Welcome of ONT, CLINIC, OCATT, BO-BARRAQUER,
Welcome of Consellería

15:30 Scientific Session I

Chairs: Dr. Blanca Miranda, Dr. Lisbeth Pels

Round-table "Kick-off" session
Discussion forum: Donor selection questions

- *Dr. John Armitage*
- *Dr. Antonio López Navidad*
- *Dr. Jesper Hjortdal*

Coffee Break

17:30 Scientific Session II
Donor selection and screening

Chairs: Dr. María Jesús Félix, Dr. Iva Dekaris

17:30 **Analysis of the factors influencing sclero-corneal buttons usefulness for transplantation**
Iwona Grabska-Liberek, Dorota Polak, Jerzy Szaflik

17:38 **Is cornea suitable for transplantation when death to preservation time is more than 14 hours?**
Cernak A., Cernak M.

17:46 **Essential elements of the physical examination in solo cornea donors**
Marja J. van Wijk, Caroline van Geyt, Audrey Laven, Hilde Beele, Arlinke G. Bokhorst

17:54 **Correlation between the duration of organ culture and the incidence of endothelial immune reactions**
P. Maier, D. Böhringer and T. Reinhard, University Eye Hospital Freiburg

18:02 **Increasing donations – getting past "why are you calling me?"**
Patricia Dahl

18:10 **Determination of corneal endothelial cell density in French Eye Banks: second look**
Delesalle N, Thuret G, Dubus J, Fleury L, Mouillot L, Gain P

18:18 **Serologic selection of cadaveric donor material according to virus B and C hepatitis at the Moscow Eye Bank**
Y.i A. Komakh

18:26 **Seroreactivity for Hepatitis B Virus, Hepatitis C Virus, Human Immune Deficiency Virus, Human T Cell Lymphoma Virus and Treponema in Iranian Corneal Donors**
Mozhgan Rezaei Kanavi MD, Kambiz Bayat Makoo, Azadeh Arayesh, Mohammad Ali Javadi MD.

18:34 **Risk analysis of NAT versus serological testing in the australian eye donor population**
Dr. Graeme A. Pollock

18:42 **Prevention of corneal endothelial cell loss during storage by anti-apoptotic gene therapy**
T. Fuchsluger, U.V. Jurkunas, A. Kazlauskas, R. Dana,.

18:50 **Adrenaline test : a new screening non-invasive method to evaluate donor cornea viability: 20-years experience**
S.A. Borzenok

18:58 **Reducing microbiologic contamination of donor globes by prolongation the diving time and different iodine contents in PVP-solution - SEM examinations of cornea endothelium of pig eyes**
Wilhelm U (Southport), Kramer A, Below H and Sietmann R (Greifswald) Hammer T (Halle), Wilhelm F and Werschnik C (HELIOS Schwerin)

20:00 Social event: Welcome reception and cocktail

Saturday 23 January 2010

08:30 Business meeting (EEBA members)

09:00 Scientific Session III
Posterior lamellar surgery
Chairs: Dr. Alfredo Adán, Dr. Rafael I. Barraquer

09:00 Highest guest lecture
Posterior lamellar surgery techniques
Dr. Mark Terry

Oral Presentations

10:30 **Femto DSEK: first trial in human with the Ziemer's LDV Femtolaser**
F. Majo MD PhD and W. Bernau CEO

10:38 **Femtosecond laser and microkeratome-assisted DSAEK versus PKP in Fuchs dystrophy and bullous keratopathy**
S. Heinzelmann, P. Maier, D. Böhringer, T. Reinhard

10:46 **A banking strategy toward customized precut corneal tissues**
Yveta Urbanova, Magdalena Netukova, Pavel Kuchynka

10:54 **The evaluation of pre-cut corneas prepared in the International Eye Bank of Prague**
Magdaléna Netuková, Yveta Urbanová, Josef Šach, Pavel Kuchynka

Coffee Break

Scientific Session IV
Limbal stem cell transplantation
Chairs: Dr. Vincent Borderie, Dr. Gilles Thuret

11:30 Keynote lecture: Techniques for *ex vivo* expansion of epithelial cells
Dr. Ricardo Casaroli-Marano

11: 40 Keynote lecture: Clinical results of *ex vivo* expansion of epithelial cells
Dr. Paolo Rama

Oral Presentations

12:00 **Molecular characterization of ex vivo expanded limbal epithelial stem cells**
Meller Da, Thomasen Ha, Pauklin Mb, Theissc C, Steuhl KPa,

12:08 **Two-step enzymatic approach for limbic progenitor cells isolation and ex-vivo expansion**
Martínez-Conesa E. Agustí E,, Vilarrodona A, Otero N, Pérez ML, Miranda B, Trías E, Casaroli-Marano RP.

12:16 **Comparison of different feeder layers for ex vivo cultivation of corneal and oral epithelium for corneal surface reconstruction**
T. Fuchsluger, S.M. Sharma, R. Dana, U.V. Jurkunas1

12:24 **Features of separation and cultivation of limbal stem cells of the eye**
*K. P. Takhchidi, S.A. Borzenok, K.D. Tonaeva, *N.A. Oniscenko, Y.A. Komakh, *M.E. Krasheninnikov, E.V. Kovshun, O.S. Volkova, O.I. Rolik, A.V. Shipunova*

12:32 **Outcomes of *ex vivo* Expanded Limbal Stem Cell Transplantarion in Humans**
F.C. Figueiredo , S. Ahmad, M. Lako' S. Kolli

12:40 **The expression of mesothelin and other mesothelial proteins in the human cornea**
Katerina Jirsova and Stanislava Merjava

12:48 **MMP-2, MMP-9, proMMP-13 and TIMP-1 in Human Cornea**
Nataša Drača, Jurica Predović,Tihomir Balog, Tanja Marotti, Maja Bohač, Ivana Romac ,Iva Dekaris

12:56 **Amniotic membrane transplantation in the treatment of persistent epithelial defect on the corneal graft**
Maja Pauk, Ivana Mravičić, Ante Barišić, Nataša Drača, Iva Dekaris

13:04 **Influence of cryopreservation and air drying on amniotic membrane and impact of storage time on cryopreserved amniotic membrane**
Thomasen Ha, Pauklin Mb, Noellec, G. Geerlingd, J. Vettere, P. Stevenf, Steuhl KPa, Meller Da

Lunch Break

15:00 Scientific Session V
Tissue specific donor selection and processing

Chairs: Dr. Blanca Miranda, Chris Stoeger

15:00 Keynote lecture: Donor selection for lamellar surgery
Dr. Juan P. Álvarez de Toledo

15:20 Keynote lecture: European Good Tissue Practices update
Dr. Esteve Trías

Oral Presentations

15:40 **Does the improvement of cataract surgery techniques increase the viable corneas for transplant?**
Otero N.; Martinez-Conesa E.; Nuñez V.; Pérez M.; Agustí E.; Vilarrodona A; Savio A.; Fariñas O.; Trias E., Miranda B.; Casaroli-Marano RP.

15:48 **Diagnosis of photorefractive keratectomy in donated whole globes**
Mozhgan Rezaei Kanavi MD. Tahereh Chamani, Mohammad Ali Javadi MD.

15:56 **Suture related complications after penetrating keratoplasty --- Advocacy for posterior lamellar grafting?**
D. Böhringer, R. Sundmacher, T. Reinhard

16:04 **Donor cornea preparation for femtosecond laser assisted keratoplasty**
Bernie Iliakis

16:12 **Clinical results of femtosecondlaser-assisted penetrating keratoplasty**
Reinhard T., Wiggermann A., Böhringer D., Maier P., Birnbaum F.
University Eye Hospital Freiburg, Killianstraße 5, 79106 Freiburg, Germany

16:20 **Preparation and shipment of endothelial grafts by the eye bank: development and validation**
Pipparelli A, Muraine M , Thuret G, Toubeau D, Lefevre S, Piselli, S, Gain P

16:28 **More efficient use of donor corneas by using Descemet Membrane Endothelial Keratoplasty (DMEK)**
Jessica Li , Esther Groeneveld- van Beek, Jacqueline van der Wees, Gerrit Melles.

16:36 **Optical and confocal microscopic evaluation of endothelial donor rolls for DMEK**
Dr. Paola Sauvageot, Dr. Ricardo Casaroli, Dr. Maria de la Paz, Dr. Juan P. Álvarez de Toledo

16:44 **Endothelial cell density after Descemet membrane endothelial keratoplasty (DMEK): 1-2 years follow-up**
Jacqueline van der Wees, Lisanne Ham, Isabel Dapena, Kyros Moutsouris and Gerrit Melles

16:52 **Visual rehabilitation following Descemet membrane endothelial keratoplasty (DMEK)**
Lisanne Ham, Isabel Dapena, Kyros Moutsouris, Gerrit Melles.

17:00 **Qualitative and quantitative parameters of pre-cut posterior corneal lamellae with stromal rim used for Descemet's Membrane Endothelial Keratoplasty with a Stromal rim (DMEK-S).**
Krabcova I. Jirsova K., Studeny P.

Coffee Break

17:30 Scientific Session VI
Workshop session

Chairs: Dr. Diego Ponzin, Dr. John Armitage

17:30 Keynote lecture: Emerging diseases. Urgent replies. Screening needs.
Scott Brubaker

18:00 Keynote lecture: Implementing the regulation of banking in the EU.
Dr. Deirdre Fehily.

Closing ceremony

20:30 Social Dinner

SCIENTIFIC SESSION I

Keynote Lectures

EEBA Donor Selection Group

W John Armitage

CTS Bristol Eye Bank, University of Bristol, Bristol, United Kingdom

A wide range of diseases have been transmitted through corneal transplantation. The purpose of the EU Tissues and Cells Directive was to unify standards for procurement, processing, preservation, storage and distribution of human tissue for transplantation within the European Economic Area. This was a welcome initiative, albeit creating challenges and difficulties for eye banks, partly owing to the air-quality requirements for the processing of tissue. However, the Directive also introduced standards for the medical assessment of donors and mandatory donor testing for certain markers of transmissible disease. It is likely that many EEBA member eye banks were already working to similar donor selection criteria by following the EEBA agreed minimum standards for donor medical assessment, which were first published as a set of contraindications to transplantation in the 2nd edition of the EEBA Directory in January 1994. These standards, termed 'agreements' since EEBA had no way of imposing them on members, were broadly based on the Eye Bank Association of America's donor medical selection criteria. In the absence of nationally developed criteria, the EEBA standards were of considerable value, in part because the criteria could be discussed at EEBA annual meetings, in the EEBA Board and, later, in the EEBA Donor Selection Group that initially comprised Diego Ponzin, Andrew Tullo, Juan Álvarez de Toledo and John Armitage. There have been continuing developments in donor selection, with often difficult questions being raised that seemingly have no clear-cut answers. In the UK in particular, the BSE epidemic and the consequent appearance of vCJD has created challenges that have driven efforts towards developing a feasible means of testing donors for the presence of abnormal prion proteins. To a large extent, the EU Directive has overtaken the EEBA standards; but it is important to remember that the EU Directive provides only minimum standards and any drives towards raising those standards or highlighting tissue-specific criteria still largely rests with member states and with organizations such as EEBA. The EEBA standards played an important role within the European eye banking community, setting criteria to minimize the risk of disease transmission by ocular tissue transplantation and thereby protecting patients from unnecessary harm. There is still an important role to play in maintaining vigilance and making sure that EEBA is consulted by the EU whenever changes to the donor selection criteria are proposed.

Corneal Transplant from donors with cancer

Antonio López-Navidad

Hospital de la Santa Creu i Sant Pau. Universidad Autónoma de Barcelona. Barcelona. Spain.

Acceptance criteria by EEBA and EBAA for corneal donation include cadavers with active cancer, both solid and hematological. Cancers excluded are: retinoblastoma, tumors of the anterior segment of the eye, primary or metastatic adenocarcinoma of the eye, leukemia, lymphoma and myeloma.

Only in three occasions the transmission of cancer by cornea transplantation has been documented: one retinoblastoma and two metastatic [adenocarcinoma and small cell, respectively] carcinoma of the eye. In the first two of them the donor presented gross masses in their eyes at retrieval and in both was affected the posterior segment of the eye. Hematological cancer transmission by corneal transplantation has never been reported.
Currently the incidence of ocular metastases is lower than 0.3% and 1.8% from solid and hematological cancer respectively. Tumor transmission through corneal transplantation is highly unlikely: metastatic dissemination is improbable in a non-vascularised tissue, the mass of neoplastic cells which could be transferred is very small; and finally, corneal recipients are immunocompetent and can prevent malignant cells dissemination. One of the possible pathogenic mechanisms that could be involved in the transference of malignant cells to the cornea is that the transmission of malignant cells from donor to recipient could take place during the process of corneoscleral excision and storage. These cells would later adhere to the inner face of the cornea in those areas deprived of endothelial cells during its storage.
Corneal donors with active cancer can represent more than 35% of corneas viable for transplantation. Ocular metastatic involvement in patients dying from active solid or hematological cancer is currently very low and transmission of malignancy is highly unlikely when there is no tumor infiltration of the eye. Then, It could be accepted any kind of active solid or hematological cancer if the following three sequential steps are fulfilled: 1) eyes which present macroscopic tumor masses should be rejected; 2) the cornea and anterior chamber of the eye should be carefully evaluated by slit lamp to discard tumor infiltration; and 3) histopathological study of the eye should be performed prior to corneal transplantation and cornea should be rejected in cases of tumor cellular infiltration.

Tissue donor selection criteria in Denmark

Jesper Hjortdal, Kim Nielsen.

The Danish Eye Bank, Aarhus University Hospital, Denmark

Along with implementation of EU-Directive 2004/23/EF in Danish legislation, supplemental NAT-testing of the donor for HIV, HBV, and HCV became mandatory in Denmark by April 2007.
The immediate consequences of these regulations were a considerable decrease in the number of collected donor corneas.

However, a new nation-wide collecting system has since then been implemented. The system is based on daily contact with most of the Danish pathological institutes and hospital morgues.
The status of all diseased patients in the Danish Central Register of Donation is checked on-line, and patients who have opted in for donation are considered for corneal donation. Final exclusion/inclusion of donors is based on patient records, physical examination of the diseased, serological testing, and NAT-testing. In addition, multi-organ donors are increasingly also being used as tissue donors, and organ donors are now routinely also NAT-tested for HIV, HBV, and HCV.

The situation has improved somewhat during 2009, but The Danish Eye Bank can at present only cover 50% of the need for corneal donor tissue in Denmark.
New initiatives related to direct employment of corneal donor tissue coordinators in the eye bank are under way.

SCIENTIFIC SESSION II

Donor selection and screening

Analysis of the Factors Influencing Sclero-Corneal Buttons Usefulness for Transplantation

Iwona Grabska-Liberek, Dorota Polak, Jerzy Szaflik

Purpose

To examine the effect of various factors such as: donor's age, cause of death, time from death to preservation and preservation length on the morphological quality of corneas used for PKP. Our purpose was to assess the role of the above factors influencing the corneal overall rating and endothelial cell density.

Methods

Sclero-corneal buttons and data concerning donors were obtained by eye bank coordinators and collected in Warsaw Eye Bank.The quality of the corneas recovered was evaluated using by Nikon NS-1V slit lamp and KONAN specular microscope.

Results

Cardio respiratory failure and cardiac arrest were the most frequent cause of the donor's death. In many cases donors with confirmed brain death who gave the corneas, were also multi-organs donors. The increasing percentage of endothelial cell loss was observed in all corneas after approximately 7 days of preservation in the medium. The mean endothelial cell density slightly decreased with donor's age, but it suggested range of the factors possibility of finding the corneas with high number of endothelial cell density both in younger and older donors.

Conclusions

The rating of the morphological state of corneas suitable for PKP depends on the length of time from death to preservation, donor's age, cause of death and time of corneas preservation. Corneas obtained shortly after the donor's death showed higher endothelial cell density and better overall rating than those removed after relatively longer period after the donor's death.An increasing percentage of endothelial cells loss was observed after 7 days of preservation irrespective of other factors.

Is cornea suitable for transplantation when death to preservation time is more than 14 hours?

Cernak A, Cernak M

Purpose

How death to preservation time influence quality of donors corneas

Methods

Donors endothelium were examined by specular microscope and also light microscope after cryopreservation and dye endothelium

Results

When death to preservation time was less than 5 hours (25 corneas) or 15 hours (50 corneas) any changes in endothelium were present. When death to preservation time was more than 15 hours (50 corneas) many endothelial cells were not vital.

Conclusion

When death to preservation time is more than 15 hours many endothelium cells were not vital

Essential elements of the physical examination in solo cornea donors.

Marja J. van Wijk, Caroline van Geyt, Audrey Laven, Hilde Beele, Arlinke G. Bokhorst.

BIS Foundation, Leiden, The Netherlands.
Tissue bank Gent, Gent University Hospital, Gent, Belgium.

Purpose

To identify the critical elements of the physical examination (PE) of potential cornea donors in order to improve the safety of cornea transplantation.

Methods

Based on literature and existing guidelines, physical signs that can indicate the presence of a contraindication mentioned in EU Directive 2006/17/EC and that could theoretically be detected during PE of potential donors, were identified. A risk assessment was designed, according to the Failure Mode and Effects Analysis (FMEA) model. Factors included were 1) prevalence of the sign in donors, 2) specificity of the sign for a contraindication, 3) severity of potential damage of the contraindication to the recipient, 4) chance of detection at PE and 5) chance of detection of the contraindication during the rest of the donor screening, as required by the EU directive. Furthermore, risks were also scored taking into consideration additional non-required control measures, such as autopsy, biopsies, cultures, anamnesis specific for detected signs, additional blood tests and additional examination of the eye at the cornea bank or at retrieval. The risks were scored, according to the worst case scenario, by three MD's, working in the field of tissue donation, including a dermatologist, supported by two quality assurance managers.

Results

74 signs associated with contraindications relevant for corneas were identified. Signs associated with advanced infection with HIV, HCV, HBV and syphilis (n=14, 18.9%) can be omitted from the PE, since these contraindications will be detected by the required serological testing. Baseline risks, taking into account the required control measures, are high for 11 signs (14.8%), associated with haematological malignancies, malignancies in the eye and risk of or early signs of transmittable disease (HIV). For 58.3% of the signs with a baseline risk higher than zero, one or more additional control measures are possible. For 45.0% of the signs specific anamnesis improves the associated risk, for 10.0% biopsy or culture, for 28.3% additional blood examinations, for 6.7% additional examination at bank or at retrieval and for 8.3% macroscopic autopsy results. Also, signs were identified, that can be omitted from PE if additional non-required tests are done, such as HTLV testing (n=4, 6.7 %). If all possible additional control measures would be taken risk priorities would remain unacceptably high for only 1 sign (1.6%), associated with haematological malignancies. However, for most additional control measures to be taken it is necessary to detect the signs at PE and for 57.1% of these signs detection at PE was difficult. Thus, the true percentage of signs associated with unacceptably high risk will probably be higher, with a maximum of 5 signs (8.3%).

Conclusions

This systematic risk assessment resulted in the identification of the minimal necessary content of the PE in potential cornea donors. Furthermore, risks associated with tissue donation were elucidated and possible risk control measures were identified and their impact was evaluated.

Correlation between the duration of organ culture and the incidence of endothelial immune reactions

P. Maier, D. Böhringer and T. Reinhard, University Eye Hospital Freiburg

Purpose

During organ culture of corneoscleral discs the number of antigen presenting cells decreases. This might result in a decrease of endothelial immune reactions with increasing duration of organ culture. To investigate this hypothesis we performed a retrospective analysis of penetrating keratoplasties that were performed during the last 5 years.

Methods

All cases of penetrating keratoplasties (n=1006) were divided into two groups regarding the median of the storage time (21 days). These two groups were compared by cox proportional hazards survival model regarding the incidence of endothelial immune reaction, clear graft survival and chronic endothelial cell loss following penetrating keratoplasty considering patient's age at time of surgery, donor's age and risk situation.

Results

We found that in the group with increased storage time statistically significantly less endothelial immune reactions occurred compared to the group with shorter duration of organ culture. However, for the duration of organ culture we did not find a statistically significant effect on clear graft survival or chronic endothelial cell loss.

Conclusion

Our results show that increased duration of organ culture leads to a lower incidence of endothelial immune reactions following penetrating keratoplasty. This might be explained by the decreasing number of antigen presenting cells in the corneoscleral discs during the course of organ culture.

Increasing donations – getting past "why are you calling me?"

Patricia Dahl

Executive Director/CEO.The Eye-Bank for Sight Restoration, Inc

Introduction

For years, awareness campaigns to increase local eye donations were focused on sharing cornea recipient success stories. The result was an increased awareness about restoring sight through cornea transplant, but not necessarily an increase in eye donations. In December 2006, The Eye-Bank for Sight Restoration embarked on a first-of-its kind multimedia ad campaign with a two-fold call to action: To "Say yes, to eye donation" and Register to become a donor today in the New York State Donate Life Registry. This campaign featured well-known actor, Jerry Orbach, who had died in 2004 and was an eye donor. This presentation will review the impact of this awareness campaign had on local donations, our public image and and enrollments in the Donate Life Registry.

Methods

The campaign was aimed at middle-income Caucasian, African American and Hispanic (not including Spanish-speaking only) adults ages 35-65, living in the five boroughs of New York City. This demographic was based on the knowledge of The Eye-Bank's average age of eye donors and the average age level and awareness level of family members who are contacted for consent. The media buy was chosen based on reaching our target audience and making impressions on more than one level of communication resulting in a combination of newspaper, television, radio and subway car advertising. With a limited budget of $150K, the ads campaign started on December 26 and ran through January, the least expensive time to advertise in NYC since it is post-holidays. Radio ads only ran again in March (which is national Eye Donor Month) and July.

Results

The number of calls received in response to the ad campaign exceeded 400 during the first campaign with the most received during the month of January and continuing through February from people wanting to enroll. Another spike of more than 300 calls occurred as a result of the July campaign. Hits to The Eye-Bank's website skyrocketed going from less than 200 per day to three times that on average. For a seven-day period from January 12 to January 18 there were nearly 8,000 hits to the Eye-Bank's website with over 1,000 on January 13 alone. Eye donations increased 35% in the first three months of 2007 and ended the year with an 8% increase. In 2008, eye donation continued to increase with a 25% increase at year-end. The need for corneas from other eye banks decrease 29% in 2007 and was about even in 2008. The Eye-Bank was able to distribute 10% and 29% more corneas in 2007 and 2008, respectively.

Conclusions

Changing the focus of our awareness campaign from recipient-based to focusing on a well-known donor has increased awareness about signing up to become a donor. The initial goal of the campaign was to enroll New Yorkers in the Donate Life Registry, but the immediate impact was recognition of The Eye-Bank for Sight Restoration and its mission. Conversations with potential donor families have been simplified because so many New Yorkers have seen or heard the ad campaign. Potential donor families readily accept and understand the purpose of our calling them after they have been notifiifed of a loved ones death. Additionally, the ad campaign has increased our visibility among New York not-profit foundations and corporations, which as proved beneficial to our fundraising efforts.

Determination of corneal endothelial cell density in French eye banks: second look

Delesalle N[1], Thuret G[2], Dubus J[1], Fleury L[1], Mouillot L[1], Gain P[2]

[1]The French Health Products Agency (Afssaps). The Laboratories and Controls Department. Unit Blood products and Cellular Therapy. Saint-Denis, France
[2]Ophthalmology department, University Hospital and Laboratory 'Biology, Engineering and imaging of corneal graft' JE2521, IFR143, University Jean Monnet. Saint-Etienne, France

Purpose

In France, approximately one cornea out of two is grafted (51% of the 8635 corneas retrieved in 2008). Among those eliminated, nearly 30% are discarded for endothelial deficiency, mainly for an insufficient endothelial cell density (ECD< 2000 cells/mm²). Considering the importance of having a precise, robust and especially reproducible ECD counting method, The French Health Products Safety Agency (Afssaps), organized from April 2008 to June 2009 a second assessment of the reliability of the routine cell count within the 18 French Eye banks.

Methods

The study design was similar to the first assessment driven by the laboratory 'Biology, engineering and imaging of Corneal Graft' in 2003 (*Transplantation* 2004; 78: 1299-1302). Five test corneas consisting of 1 mm^2 of flat mounted, fixed and alizarin stained human corneal endothelium (ECD: 825, 1185, 2047, 2262, and 2952 cells/mm^2, determined by 2 calibrated methods) were selected and successively sent to the 18 Eye banks, all volunteer to participate. All the usual technicians of each bank had to count the test corneas using the routine method(s) employed to assess grafts. In addition, they also had to assess cell morphometry. A questionnaire enclosed with the slides aimed to record the details of cornea preparation methods used for endothelial control (organ culture medium, technique for cell visualization, counting method itself, microscope type including calibration factor, computerized image analysis system if necessary)

Results

Two successive endothelial controls (beginning and end of storage) were carried out by 89% (16/18) of the banks. A manual counting technique was used to validate the corneas in 72% (13/18) of the cases and a computerized image analysis system (4 different systems) in 28% (5/18). The cornea preparation methods before counting remained heterogeneous. The fixed frame counting technique was used by 72% (13/18) of Eye Banks. The classical manual technique (cells counted in 5 squares of a reticule placed in the microscope eyepiece) was used by 39% of banks. Only 83% (15/18) calibrated their microscope, and 53% of them did not have a correction factor for counting. For the five test corneas: 430 counts were carried out by 70 eye banks technicians, by manual and/or image analysis system. As result on ECD determination, 42% (180/430) deviated by more than 10% from the expected ECD. Among them, 128 were over-estimated (maximal difference +87,9%) and 52 were under-estimated (maximal difference -31,4%). Two banks constantly over-estimated (in the mean +31,7% and +42,7%, no calibration and/or material problem) but the 16 other banks were in average within ±13% from expected ECDs. For manual methods, a statistically significant difference between banks was observed for the five test corneas, whereas no difference was observed with image analyzers. ECD obtained with the analysers were closer to expected values than with the manual methods. Compared to the study done in 2003, reliability of ECD determination globally improved.

Conclusions

ECD measurements appear acceptable in most of the French Eye Banks. Only two of them must improve their counting methods and/or solve a material problem. Methods of corneal preparation before endothelial quality assessment as well as strategies for cell counting and morphometry determination should be standardized. Once again, image analysis systems prove more reliable (precise and with a lower intra and inter observer variability) than manual counting methods. This new assessment ('second look') of Eye banks will allow editing recommendations to improve ECD determination.

Serologic selection of cadaveric donor material according to virus B and C hepatitis at the Moscow Eye Bank

Y.i A. Komakh, The Fyodorov Eye Microsurgery Federal State Institution, Moscow, Russia

Introduction

Within 2008 the receipt of corneas from seropositive donor cadavers infected with B and C hepatitis was 14.9% at the Moscow Eye Bank. Therewith the average periods of cadaveric corneas selection increased also from 16 to 24 hours.

Purpose

To determine maximum permissible time after donor death that does not influence appearance of false seropositive reactions.

Material and methods

Serological diagnosis was performed by two ELISA tests: screening and confirmative. PCR DNA-HBV and RNA-HCV verifications were carried out parallel. There were analyzed 245 seropositive donors.

Results

In periods up to 12 hours after death (n=36 cadavers) ELISA seropositive results coincided with RNA-HCV and DNA-HBV results in 100% of cases. In periods from 13 to 16 hours (n=74 cadavers) coincidence of HBV was 100%, HCV – 88%. In periods from 17 to 24 hours (n=135 cadavers) coincidence of HBV was 92%, HCV – 74%.

Conclusions

In periods after death of donor cadavers up to 12 hours the false-positive serological reactions are not observed, after 13 hours the false-positive serological reactions should be verified by means of PCR testing to avoid unwarrantably great number of qualitative donor cornea rejection. However, in Russia the highly sensitive PCR test is still only auxiliary method and is not allowed for examination of donor cadavers without the main method of ELISA diagnosis.

Seroreactivity for Hepatitis B Virus, Hepatitis C Virus, Human Immune Deficiency Virus, Human T Cell Lymphoma Virus and Treponema in Iranian Corneal Donors

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Purpose

To determine seroreactivity for Hepatitis B surface antigen (HBS Ag), Hepatitis C virus (HCV), Human immune deficiency virus (HIV), Human T cell lymphoma virus (HTLV) and Treponema among corneal donors in the Central Eye Bank of Iran.

Methods

All the eye bank records of seroreactivity for HIV, HBS, HCV, HTLV and Treponema among corneal donors between 1996 and 2008 were reviewed. Linear regression model was employed statistically for each type of seroreactivity considering their frequencies.

Results

Serologic data of 24484 corneal donors were reviewed. Seroreactivity in order of frequency included HCV in 3.6% (n=899), HBS Ag in 3.2% (n=785), HIV in 0.5% (n=134), HTLV in 0.3% (n=78), and Treponema in 0.1% (n=27). Multiple seroreactivity was found in 110 donors. Seroreactivity for HBS Ag showed a decreasing trend (P value=0.003) using regression analysis. Statistically, no significant change of trend was noted in seroreactivity for HCV, HIV, HTLV and Treponema (P value=0.092, 0.679, 0.203 and 0.143 respectively).

Conclusion

Significant decreasing trend in seroreactivity for Hepatitis B virus and lack of the change of trend for HCV, HIV, HTLV and Treponema seroreactivity among corneal donors in Central Eye Bank of Iran might be partially contributed to the increased public knowledge about transmissible viral diseases and possible increased rate of vaccination for Hepatitis B virus.

Risk analysis of NAT versus Serological Testing in the Australian Eye Donor Population.

Dr Graeme A Pollock

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Purpose

Serological testing of eye donors for Human Immunodeficiency virus (HIV), Hepatitis B virus (HBV) and Hepatitis C virus (HCV) has been standard eye banking practice for a number of decades. Since its routine implementation there have been no reported cases of transmission of these viruses by corneal transplantation. More recently, Nucleic Acid Amplification Technology (NAT) testing of donors has been mandated by many nation's regulatory authorities as it offers a reduced "window period" between infection and detection of these viruses. However, the residual risk associated with NAT testing of eye donors as compared to serological methods is seldom considered within the context of prevalence and incidence of a virus within an Eye Bank's regional donor pool. In addition, the selection of an appropriate testing regime should also take into account the potential for transmission of a virus via corneal transplantation and the availability, efficiency and cost of the tests. This paper provides an estimate of residual risk in the Australian eye donor population, compares the results to other tissue banking and eye banking jurisdictions both nationally and overseas, and discusses the results in the context of cost versus benefit.

Methods

Prevalence of the three viruses in the Australian eye donor population were determined from the Eye Bank Association of Australia and New Zealand data on reactive serology for HIV, HBV and HCV from September 2007 - June 2009 (from 1,875 contiguous donations). Incidence of the viruses in the eye donor population was calculated by extrapolation of prevalence and incidence data on Australian blood donors, and residual risk estimated using an Incidence-window period mathematical model $P=\lambda \times WP$ where P = probability donor gave infectious donation during window period, λ=the incidence, WP=window period (in days).[1] The cost of NAT testing was based on the current Australian average of $150 for all three viruses (€93).

Results

Prevalence in the eye donor population for HIV, HBV and HCV was 5.93, 155.17 and 271.55 per 100,000 persons respectively. Incidence for HIV, HBV and HCV was calculated at 0.35, 1.29 and 3.02 per 100,000 person years respectively. Residual risk (i.e. odds of an infected donor not being detected by the selected testing method) were for serologic testing: HIV 1 in 4,739,336, HBV 1 in 479,613 and HCV 1 in 172,651. NAT testing reduced residual risk to: HIV 1 in 14,897,959, HBV 1 in 1,414,728 and HCV 1 in 1,726,584. At the current Australian eye donor rates the probability and the cost of detecting a virus by NAT that *has not* been detected by serological methods are HIV – 1 every 4,739 years at $237m (€147m), HBV - 1 every 479 years at $24m (€15m), HCV - 1 every 172 years at $8.6m (€5.3m).

Conclusions

Prevalence rates of HIV, HBV and HCV in the Australian eye donor population are similar to the Australian blood donor population. While these results show that implementing NAT to screen individual eye donors reduces the residual risk of a donor being viraemic the benefit is marginal. The reduction of an already very low risk, the high cost, limited availability and limited routine turn-around times of cadaveric NAT testing across Australia, make the introduction of mandatory NAT testing of eye donors unsupportable.

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Prevention of Corneal Endothelial Cell Loss during Storage by Anti-Apoptotic Gene Therapy

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Purpose

Regardless of the inciting cause, CEC loss is a common denominator of corneal graft failure. CEC loss *during storage* results in significant loss of suitable tissue for grafting, CEC loss *after transplantation* is a major cause of graft failure. The purpose of these studies is to investigate the role of apoptosis in CEC in order to prevent CEC loss during storage and transplantation.

Methods

Gene transfer of Lenti-Bcl-xL or –p35 was accomplished in human donor corneas, primary cultured CEC and an immortalized CEC line and compared to untreated controls. Cell death (apoptosis) was induced by Actinomycin or Etoposide (external vs. internal apoptotic pathway, respectively). In addition, CEC loss during preservation was studied both during Optisol GS (4C) and organ culture storage (37C, Biochrome Medium I). Both storage media were diluted with PBS to promote cell loss. CEC were enumerated, apoptosis was detected by TUNEL staining and confocal microscopy.

Results

The percentage of TUNEL-positive CEC provoked by the apoptotic inducers was significantly reduced relative to controls. Transfected corneas preserved an almost intact endothelial monolayer while controls nearly entirely lost vital CEC. During long-term storage experiments at 4C and at 37C, CEC counts in corneas expressing anti-apoptotic genes were significantly increased compared to the controls.

Conclusions

Protection of CEC by anti-apoptotic genes appears to be an effective method to reduce CEC loss during storage. The application of this technique could increase the amount of high quality grafts in eye banking and further reduce graft failure following corneal transplantation, and is be of specific interest as to precut corneas and DSAEK procedures.

Adrenaline test: a new screening non-invasive method to evaluate donor cornea viability: 20-year experience

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Introduction

A morphometric characterization of cadaveric corneal endothelium is not an absolute and single criterion of donor material selection for penetrating keratoplasties. Morphologic component should be supplemented with a functional criterion of viability, namely, an evaluation of residual of macro-energetic compounds in endothelial cells after donor death. Twenty years ago we have developed and introduced into practice of the Moscow Eye Bank a simple and safe method of cornea viability evaluation – adrenaline test. Recently we showed that the biological result of penetrating keratoplasty was greatly determined by residual quota of ATP in cadaveric corneal endothelium. A screening non-invasive method was suggested - adrenaline test.

Purpose

To develop an adequate functional criterion of viability for human cadaveric corneas – adrenaline test.

Materials and methods

During 20 years 23000 cadaveric donor corneas were tested. However, at the first stage of the investigations we evaluated 120 donor eyes by means of the offered adrenaline test and ^{31}P-NMR-spectroscopy. Results of adrenaline test were verified also by method of clinical transplantology – penetrating subtotal keratoplasty (n=1000 recipients).

Results

In accordance with the latent period from instilling of 0.1% solution of adrenaline hydrochloride to the beginning of mydriasis (the phenomenon of “the cat eye”) on the cadaveric donor eye, we judge about the residual quota of ATP in endothelium. Latent period up to 5, 10 and 15 min. implies the lack of ATP equal to 0-55, 56-69 and 70-100 relative %, respectively. The indices of the adrenaline test are well correlated with the results of ^{31}P-NMR-spectroscopy of the donor corneas; the index up to 5, 10 and 15 min. paralleled ATP content equivalent to 7.00 - 6.24, 6.23 - 6.05 and 6.04 - 5.63 μM per gram of humid weight of the cornea, respectively. There is a slight correlation ($r=0.207$, $p<0.05$) between the index of adrenaline test and postmortal period (6 to 18 hours). Perhaps, it indicates the peculiarities of premortal energetic metabolism of the donor and his tanatogenesis. Clinical method - the transplantation itself - proves that the index of the adrenaline test influences the biological result of keratoplasty, that is the viability of the donor transplant ($r=0.484$, $p=0.000$).

Conclusions

Today, on the basis of the great number of clinical data, it was shown that the donor material is viable when the results of the adrenaline test are not more than 10 min. (residual quota of ATP in cells of cornea >30 relative per cent).

Reducing microbiologic contamination of donor globes by prolongation the diving time and different iodine contents in PVP-solution - SEM examinations of cornea endothelium of pig eyes

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Background

Decontamination of human donor globes in PVP-Iodine solution before the transplantation is an established method. Unfortunately there are still transplants loosed due to microbial contamination of organ cultures. Increasing the decontamination time and iodine concentration could result in a more effective microbial elimination. The question now is: Will this cause any damage to the cornea endothelium? Using the scanning electron microscope, the cornea endothelium of pig eyes was observed for changes depending on the decontamination time and the iodine concentration within the PVP-Iodine solution.

Methods

Each experimental trial version consisted of 10 pig eyes, which are exposed for 5; 10; 30 minutes to either 100% or 50% concentrated PVP-Iodine solution. Following this the corneas were gently removed and prepared for the scanning electron microscopic examination. By using a score the endothelium was semi quantitative evaluated. Finally the trial versions were compared to each other.

Results

Regarding to trial version zero (with out exposure to PVP-Iodine solution) was the cornea endothelium exposed for 5 and 30 minutes of diving in PVP-Iodine solution.
By increasing the iodine concentration only a score of a few points could be given.

Conclusion

The examination of pig eyes corneal endothelium using a scanning electron microscope demonstrated, that prolongation the exposure time to 10 minutes and increasing the iodine concentration in PVP- Iodine solution will not cause damage to the cornea endothelium. Further experiments, in which the eye balls are contaminated with highly virulent microbes, will be necessary to show the improvement of the decontamination by elongating the exposure time and increasing the iodine concentration.

SCIENTIFIC SESSION III

Posterior lamellar surgery

Highest Guest Lecture

Posterior Lamellar Surgery Techniques

Mark A. Terry, M.D.

Over the past 10 years, endothelial keratoplasty (EK) has gone from an experimental laboratory procedure with unknown risks to the preferred method of therapy for the treatment of patients suffering from corneal endothelial dysfunction. Selective replacement of the endothelium through posterior lamellar transplantation was first performed by Charles Tillet in 1956 using mattress sutures to attach the donor tissue. However, in 1998, Dr. Gerrit Melles made a breakthrough discovery that donor tissue could be successfully attached using only a temporary air bubble for support, and this opened the modern era or posterior lamellar keratoplasty (PLK). In March of 2000, after a year of further laboratory development of PLK, Dr. Mark Terry performed the first successful PLK procedure in the United States, the second surgeon in the world to successfully perform this form of surgery, and he named this revised surgery deep lamellar endothelial keratoplasty. (DLEK) Although allowing much better results than standard full thickness PKP surgery, the PLK/DLEK techniques involved manual deep lamellar dissections of the cornea, were difficult for most surgeons and general acceptance was minimal. Dr. Melles again revised the surgical technique to eliminate manual dissections of the recipient with only stripping of the diseased Descemet's membrane, and he published the first clinical results of Descemet's stripping EK (DSEK) in about 2004. The technique of DSEK, when combined with microkeratome preparation of the donor tissue was termed DSAEK and because of the ease of surgery and the readily available tissue that was "pre-cut" by the eye banks, DSAEK has now become the dominant form of EK surgery throughout the world. Drs. Mark Terry and Frank Price have separately published results on the largest series of DSAEK cases in the world and have reported extensively on the risks and benefits of this form of surgery. The visual results of Terry et al after DSAEK report that >95% of eyes achieve 20/40 or better visual acuity after DSAEK and >20% achieve 20/20 or better. Most importantly, the complications from DSAEK using the published Terry Technique of this surgery were the lowest in the world with a 1.8% rate of dislocation and 0% rate of graft failure or replacement.

DSAEK surgery involves the stripping of the diseased recipient Descemet's membrane followed by the insertion of a posterior lamellar donor corneal disc onto the stripped recipient bed. The insertion of the tissue is the most variable of the steps of the various DSAEK surgery techniques. Although most insertion techniques involve passage of the folded tissue through a 5 mm limbal incision, some techniques use a small 3 mm incision. Both laboratory and clinical data now prove that the compression of the donor tissue causes substantial damage to the donor endothelium, with the smaller the incision for insertion, the greater the damage to the endothelium. Insertion of the donor tissue can be accomplished using a forceps for direct insertion, a suture or forceps from the distal limbus as a "pull through" method, or an instrument that acts as a "glide" to either roll the tissue without folding and/or ease the delivery through the incision. Current published data on endothelial cell loss from these various techniques of insertion demonstrate no difference in donor cell loss, emphasizing again that incision size is the dominant determinant of tissue damage. Most recently, tissue delivery devices have been introduced which may eliminate the compressive forces which occur from the wound, allowing even greater donor endothelial survival and graft health.

The most recent iteration of EK is the pure anatomic replacement of Descemet's membrane with the procedure introduced by Gerrit Melles in 2006 called Descemet's membrane EK (DMEK). (Most recently, Frank Price and Mussimo Busin have worked to develop a hybrid form of DMEK called Descemet's membrane automated EK (DMAEK) which leaves a stromal rim of tissue to improve adherence of the donor tissue but there are not published series on the results of this hybrid.) The DMEK procedure is attractive due to the purported earlier visual recovery of patients as well as the higher percentage of eyes that reach the level of 20/20 vision. However, the most recent large series (n=60) by Price et al in 2009 demonstrates that the price of marginally improved visual results is the increase in re-operation rates of 63% for re-bubbling and 8% for graft failure and replacement. In addition, the challenges of donor preparation of DMEK tissue can result in a substantial proportion of donor tissue wastage (8%). A donor tissue wastage rate of greater than 5% would make the prospect of technician prepared DMEK donors for "pre-cut" tissue untenable. While DMEK is a promising technique for endothelial transplantation, further refinement needs to be done to make this technique safer for the patient and financially viable for the eye banks and surgeons before general acceptance is anticipated.

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Oral Presentations

Femto DSEK: first trial in human with the Ziemer's LDV Femtolaser

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Purpose

To report the first case of Femto DSEK with the Ziemer's LDV Femtolaser.

Methods

A 66 years old woman with a pseudophakic dystrophy of her right eye was operated. To prepare the donor, we used a new anterior artificial chamber with a new head for the LDV femtolaser able to cut at 500-micron meters deep. New software for the LDV has also been developed for this application. On the recipient, the procedure was equivalent to the technique we currently use to perform DSAEK. Follow-up included BCVA, corneal thickness and transparency, and adherence of the graft on the recipient.

Results

The anterior chamber was efficient to maintain a sufficient pressure during the cut of the donor. The new head of the laser was able to perform a deep, regular and smooth cut. The thickness of the donor was about 90-micron meters. Adherence of the lamella was good and after a follow-up of 3 months, the final BCVA was 20/32.

Conclusion

This report is a proof of principal, that the LDV femtolaser can be used for posterior lamellar grafts. Survival of the endothelial cells compared to the DSAEK remains the important point to validate the usefulness of this new technique.

Femtosecond laser and microkeratome assisted DSAEK versus PKP in Fuchs Dystrophy and Bullous Keratopathy

S. Heinzelmann, P. Maier, D. Böhringer, T. Reinhard

Purpose

Until recently, penetrating keratoplasty (pKP) was the only option to restore vision in Fuchs endothelial dystrophy and bullous keratopathy. This procedure is effective but may induce considerably amounts of corneal astigmatism. Descemet stripping automated endothelial keratoplasty (DSAEK) is a new therapeutic option. This approach has the advantage of not requiring open sky procedures and graft suturing. Therefore it can be performed in local anaesthesia. Near visual acuity sufficient for reading may be achieved within the first postoperative weeks. Optical performance beyond, however, is potentially degraded from a stromal graft host interface in the optical zone. We compared precut versus directly cut grafts as well as femtosecondlaser versus microkeratome assisted preparation of the posterior lamellae.

Methods

Until now we performed 42 DSAEK procedures using either the femtosecond laser or a microkeratome for the preparation of the posterior lamellae. Donor preparation took place at the eye bank 1 day before surgery or in the operation theatre during surgery. Handling and positioning of the graft was accomplished as previously described. The control group consisted of 80 penetrating keratoplasties, where a guided trephination system was used.

Results

The clinical observations revealed good anatomical postoperative results with low astigmatism and a slight hyperopic shift following DSAEK. However, visual acuity (20/200-16/20) remained low in comparison to pKP (1/20-20/20). Complications were dislocation or failure of the graft, glaucoma and rejection. Femtosecondlaser- and precut tissue lead to diminished visual outcome compared to mechanical and direct preparation.

Conclusions

According to our data, the new techniques of Descemet stripping automated endothelial keratoplasty seem to provide fast visual rehabilitation but a lower longterm visual acuity compared with pKP. The optimal preparation time point seems to be directly insertion of the graft after cutting. Furthermore, microkeratome-assisted DSAEK provided better visual outcome than femtosecondlaser-assisted DSAEK. To further investigate the effectiveness of this new surgical approach we propose a randomized clinical trial to compare visual outcome of pKP to DSAEK.

A Banking Strategy toward customized Precut corneal Tissues

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Purpose

To introduce eye tissue bank strategy for advanced lamellar program utilizing the Moria Microkeratome system.

Methods

Eye Bank OTB01 has assured compliance with the new Czech national regulations for Clear Room environment to ensure further processing of donor corneal tissue. The Moria ALTK-CBM System has been supplied with the other required instrumentation utilized in processing donor corneal tissue within the advanced lamellar program. The advantage of the educational program for DSAEK comprehensive training was organized by the National Eye Bank Center in Memphis, Tennessee, which is a state of the art facility, established by Tissue Banks International. Two Eye Bank delegates were trained during a comprehensive two week program in preparation for the proper utilization of the Moria Microkeratome System.

Results

Successful outcome of the comprehensive DSAEK training program resulted in the certification of both Eye Bank delegates for use of the Moria Microkeratome System. The evaluation, including slit lamp microscopy, specular microscopy, and utilization of the Visante OCT has confirmed proper precut technique with very good results. The processing of corneal tissue for DSAEK surgeries has been developed into a standardized method and has been well accepted by Czech surgeons. In addition, several wet labs courses were organized by corneal surgeons and Eye Bank staff to further the education of the advanced lamellar program.

Conclusions

We believe precut corneal tissues prepared in our Eye Bank by the National Eye Bank Center-Tissue Banks International standardized method has become a great method for processing safe donor tissue within the Eye Bank environment. OTB01 has demonstrated to be one of the few tissue banks providing the highest quality corneal tissue by a certified Eye Bank team.

The evaluation of Pre-Cut corneas prepared in the International Eye Bank of Prague

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Purpose

To evaluate quality of Pre-Cut tissues prepared in our Eye bank and analyse possible complications during and after Pre-Cut cornea preparation.

Methods

30 Pre-Cut corneas were prepared from donors which were not suitable for surgery. Lamellar grafts were prepared using 300um Moria microceratome. Methods for preparation were adopted form International Federation of Eye and Tissue Banks – Tissue Banks international guidelines. After preparation precise Slit lamp evaluation, Specular microscopy evaluation and in some cases histological staining were performed.

Results

Mean cell density prior cut was 2709±483(SD) cells/mm^2, and 2623±407(SD) cells/mm^2 after preparation. We had no statistically significant endothelial cell loss after preparation. In two cases we found lamellar dislocation into the Eusol medium. All lamellas were clear after 1 week, no medium showed signs of bacterial contamination.

Conclusions

We believe that preparation of Pre-Cut corneas in our eye bank provides tissues of high quality and safety. And we also believe that in the near future Pre-Cut corneas will represent majority of tissues provided by our eye bank

SCIENTIFIC SESSION IV

Limbal stem cell transplantation

Keynote Lectures

Techniques for ex vivo expansion of epithelial cells

Dr. Ricardo Casaroli-Marano

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The use of progenitor cells with regenerative and restorative purpose of organs and tissues is a current subject of important scientific interest. The first effective clinical results in regenerative medicine through cellular therapy were applied to the treatment of patients with large areas of burned skin surface. Specifically, thanks to procedures developed to produce epithelial sheets from cultures of epidermal keratinocytes isolated from human skin and expanded ex vivo. From this previous experience in the field of dermatology, the epithelial cells of the cornea have been obtained through cell culture techniques to be expanded ex vivo. Later, it could successfully reconstruct the human ocular surface by the use of progenitor cells from sclero-corneal limbus (LSCs) in patients with severe unilateral illness of ocular surface. The ex vivo expansion of LSCs is the newest method for ocular surface restoration. From minimally invasive biopsy of healthy limbal region (contralateral eye or the same eye), the epithelial layer is separated by an enzymatic treatment. The cells obtained are co-cultured in vitro, using cell culture techniques on feeder layers (irradiated or mitomycin-C inactivated 3T3 fibroblasts). Once cell culture achieved growth by obtaining epithelial sheets, they can be transferred to suitable substrates such as amniotic membrane, fibrin, or biocompatible polymers. This methodology has numerous advantages over the limbal transplantation techniques employed so far. Essentially, this approach requires a substantially smaller limbal biopsy, which mitigates the risk of induction of limbic deficits in healthy donor tissue. Another theoretical advantage would be the reduced risk of allograft rejection due the absence of the antigen presenting Langerhan's cell in ex vivo cultures obtained. It is always desirable to use autologous cells for ex vivo expansion to avoid the risk of immune rejection. However, with this method in the presence of bilateral ocular involvement, it would be acceptable to use heterologous epithelial cells or an alternative source of autologous healthy epithelial cells. Heterologous epithelial cells can be obtained from cadaver or living related donor corneas.

Long-term results of Autologous Fibrin-Cultured Limbal Stem Cells Transplantation

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Purpose

To evaluate the long-term outcome of autologous fibrin-cultured limbal stem cells transplantation.

Methods

Patients with limbal stem-cell deficiency were grafted with autologous limbal stem cells cultivated on 3T3-J2 and fibrin.

Results

Between 1998 and 2007 we performed 125 procedures on 112 patients. One patient was grafted in both eyes, 11 patients were grafted more than once. Six patients were lost. The overall results, after one or more procedures, were: 76.64% success, 13.08% partial success and 10,28% failure. No failures were reported after one year.

Conclusion

The procedure appears safe and stable over time.

Oral Presentations

Molecular characterization of ex vivo expanded limbal epithelial stem cells

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Purpose

Transplantation of limbal epithelial stem cells (HLECs) expanded on amniotic membrane (AM) is a new surgical approach to reconstruct ocular surface in limbal stem cell deficiency. However, it is still unknown if ex vivo expanded limbal epithelial cells retain their stem cell characteristics during cultivation. Therefore, it was our goal to assess stem cell properties of HLECs cultivated on AM.

Methods

HLECs isolated from donor corneas were ex vivo expanded on intact AM and plastic and analyzed by means of biochemical, cell biological and molecular biological methods. Label retaining cells were analyzed using BrdU labelling. Formation and functionality of GAP-junctions were studied by analysis of connexin 43 expression and dye transfer experiments. The effect of tumor promoting substance PMA on the cellular phenotype and cell cycle kinetics was measured. The expression pattern of markers for stemness (ABCG2, p63), corneal epithelial differentiation (K3, K12) and of several markers for pluripotency (among others OCT4, SOX2 and NANOG) in cultivated HLECs was studied by means of Real-Time PCR.

Results

HLECs cultivated on AM display label retaining comparable to HLECs in situ and form significantly less GAP junctions compared to HLECs grown on plastic. The expression pattern of HLECs in situ and expanded on AM differs from each other, nevertheless stem cell and pluripotency markers are expressed in expanded HLECs.

Conclusions

HLECs expanded on AM retain major stem cell characteristics and are therefore suitable for corneal surface reconstruction in limbal stem cell deficiency.

Two-step enzymatic approach for limbic progenitor cells isolation and ex-vivo expansion.

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Purpose

The use of stem cells is promising for future cell-based therapies such as tissue regeneration and engineering. In ocular surface pathologies, transplantation of autologous or heterologous corneal stem cells could be applied in several cases in which unilateral or bilateral disease produces important corneal stem-cell deficiency. We describe methodology to obtain sclero-corneal progenitor stem cells from human limbus and their expansion ex-vivo.

Methods

Two-step enzymatic method (dispase/trypsin) was carried–out. The first step was realized at low (4º) and the second at high temperature (37ºC), in order to obtain the epithelial sheets and the cellular suspension respectively. Co-cultures on mitomycin-C 3T3-SA inactivated feeder-layers were used to expand limbic epithelial cells. The characterization of cellular population was performed by using several markers, such as p63, cytokeratins (CK) as well as E-cadherin. Quantitative real-time PCR (qPCR) was used to study epithelial and progenitor origin of the cell cultures obtained. Protein pattern expression was analyzed by SDS-PAGE and Immunoblot experiments. Corneal epithelial (HCE) cells immortalized with Simian Virus 40 were used as control for specific markers.

Results

Large epithelial sheets of limbic cells could be isolated with dispase treatment (O/N, 4ºC). Epithelial cell separation was easily performed with trypsin treatment (30 minutes, 37ºC). Cellular suspensions in feeder-layer co-cultures showed clonal growth pattern after 48-72 hours. Experiments realized by qPCR showed high expression of ΔNp63α, CK3 and CK12 in limbic progenitor cells when compared with mRNA expression in HCE control cells. The protein expression of progenitor cells was also confirmed by Western-blot analysis and showed higher expression of p63, CK3/12 and CK1/5/10/14 than control cells. E-cadherin and CK19 expression were found similar in both cell lines.

Conclusions

Two-step enzymatic method has been optimized in our laboratory and can be considerate useful to isolate progenitor cells from human limbus. Feeder-layer techniques for ex-vivo expansion allowed us to maintain undifferentiated cellular characteristics for application in cell therapy approaches.

Comparison of Different Feeder Layers for *ex vivo* Cultivation of Corneal and Oral Epithelium for Corneal Surface Reconstruction

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Purpose

To investigate the propagation of e*x vivo* cultivated oral and corneal epithelial cells on amniotic membrane with and without different feeder layers, and to compare the cell sheet characteristics between these different culture conditions.

Methods

Oral and limbal epithelial cells were isolated by microdissection followed by digestion with dispase and trypsin/EDTA. The cells were seeded on intact amniotic membrane and co-cultured with (1) mouse 3T3 fibroblast (2) human dermal fibroblast (3) human bone marrow fibroblast feeder layers, and (4) with no feeder layer, for three weeks. The cultivated sheets were characterized by immunofluorescence with epithelial, stem cell, and cell junction markers. In addition, colony forming assays of the cultivated epithelia were established to evaluate stem cell properties.

Results

The cultivated sheets from corneal and oral mucosa specimens formed stratified epithelium. There were no discernible morphological differences between cells grown with and without feeder layers for both oral and corneal cells. Immunohistochemistry showed positive staining for cytokeratin-3, cytokeratin-4 and connexin 43, differentiation markers for corneal epithelium, and integrin-ß1, putative stem cell marker, in all epithelial cells, including the ones grown without a feeder layer. Co-cultivation of limbal epithelium with 3T3 cells leads to the highest expression in studied markers.
For oral mucosa, though, co-cultures with bone marrow or dermal fibroblasts lead to highest expression of the studied antibodies. Colony-forming efficiency in oral mucosa co-cultured with bone-marrow fibroblasts was up to 9%.

Conclusions

Stratified epithelial sheets of oral and limbal epithelium expressing putative stem cell and corneal epithelial markers were successfully cultured on amniotic membrane with alternative human-derived feeder layers. Interestingly, our culture conditions were conducive to epithelial cell growth and differentiation even without a feeder layer. Further experiments are required to assess the suitability of these cell sheets for human transplantation.

Features of separation and cultivation of limbal stem cells of the eye.

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Introduction

Stem/progenitor cells have an ability to optimize the cytocine status in the cellular microenvironments after their local co-transplantation in case of organ and tissue transplantation. There are available isolated publications about combined transplantations of human donor corneas and allogenic limbal stem/ прогениторных cells (LSC) after their preliminary cultivation in clinic in the literature. Therewith a technology of allogenic limbal cells separation, cultivation from donor cadavers and verification of their immune suppressive properties for clinical use are not developed.

Purpose

To develop a technology of allogenic human LSC separation and cultivation for co-transplantation with donor cornea.

Methods

Palisades of Vogt with the most LSC quantity was separated from 16 human cadaver donor eyes. The LSC separation was performed by the trypsinization method using collagenases of types I-III and EDTA. We used for cultivation the DMEM/F12 medium containing fetal bovine serum and a number of growth factors.

Results

Within the follow-up a proliferative LSC activity was observed independent of the separation method. Cells were in form of loose conglomerates in a suspended condition and exhibited a tendency to colony formation. The absence of cell adhesion at the first stages of cultivation was caused by a low floatable density of limbal stem cells and a presence of a high collagen concentration in the medium.

Conclusions

The task of further investigations is a search of optimal methods for LSC cultivation in a sufficient volume for application in clinic in case of co-transplantation with donor cornea.

Outcomes of *ex vivo* Expanded Limbal Stem Cell Transplantation in Humans

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Purpose

To study the clinical outcome of transplanting animal-free cultivated limbal epithelial cells on human amniotic membrane (AM) for the treatment of patients with limbal stem cell deficiency (LSCD).

Methods

Prospective, single-centre, noncomparative, interventional case series. Participants: eight eyes of 8 consecutive patients with unilateral LSCD (chemical burns in 6 eyes, thermal burn in 1 eye and radiation keratopathy in 1 eye) were treated at the Department of Ophthalmology, Royal Victoria Infirmary, Newcastle upon Tyne, UK between April 2006 and November 2009. Intervention: autologous limbal epithelium from the healthy contralateral eye was cultivated on AM without the requirement of animal cells or products, and the expanded epithelium was transplanted onto the ocular surface of the eye with severe LSCD. Main outcome measures: ocular surface reconstruction with corneal epithelialization, absence of superficial corneal vascularisation, change in visual acuity, absence of conjunctiva-derived goblet cells on corneal impression cytology (IC) and postoperative complications were all studied. Patient-reported outcomes including vision impairment and pain/discomfort scores were also recorded.

Results

Seven of the patients were male and the mean age was 43 (range 16 - 73). Mean follow up was 18 months (range 8 - 31). Postoperatively, satisfactory ocular surface reconstruction was obtained in all eyes (100%), as confirmed by IC. However, three of the eight eyes developed localised conjunctival invasion, requiring subsequent sectoral epitheliectomy. Penetrating keratoplasty was performed in 1 eye. At last examination, visual acuity improved in 5 eyes and remained unchanged in 3 eyes. Vision impairment and pain scores improved in all patients ($p<0.05$). Complications: ocular surface exposure that required lid surgery in 1 eye and corneal graft rejection in 1 eye.

Conclusions

This study demonstrates that transplantation of autologous limbal epithelial stem cells cultured on amniotic membrane without the use of animal cells or products is an effective method of reconstructing the corneal surface and restoring useful vision in patients with unilateral LSCD. This procedure has the potential to become a viable management option for patients with severe LSCD but it requires more resources. Further studies with more patients and longer follow-up should be conducted.

The expression of mesothelin and other mesothelial proteins in the human cornea

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Purpose

To determine whether proteins that are characteristic of the human mesothelial cell phenotype such as mesothelin, proteinase inhibitor-9 (PI-9), calretinin and HBME-1 protein, are expressed in human corneal endothelium.

Methods

Five cadaverous human corneo-scleral discs were used. The detection of PI-9, mesothelin, calretinin and HBME-1 protein (a membrane protein of mesothelial cells) was performed on cryosections and corneal endothelial imprints using indirect immunofluorescent or enzymatic immuncytochemistry. The staining intensity was assessed using fluorescent or light microscopy, and the percentage of positive cells was calculated from at least two hundred cells. Semi-quantitative RT PCR (RNA was isolated from endothelial imprints) was performed for the detection of PI-9 and mesothelin mRNA.

Results

PI-9 was present in most of the corneal endothelial cells. The strong and week signal for mesothelin was present in the epithelium and endothelium respectively. The expression of PI-9 and mesothelin was determined at the mRNA level. Calretinin was detected in the corneal epithelium and less intensively in the corneal endothelium, where both cytoplasmic and nuclear localisation was demonstrated. HBME-1 antibody strongly stained the corneal endothelium and stromal keratocytes. Marked positivity was present in the stromal extracellular matrix, while no staining was present in the sclera.

Conclusions

We detected PI-9, mesothelin, calretinin and HBME-1 protein in human cornea, particularly in the endothelium, confirming that this layer expresses markers typical of mesothelial cells.

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MMP-2, MMP-9, proMMP-13 and TIMP-1 in Human Cornea.

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Purpose

Keratoconus (KC) and bullous keratopathy (BK) are causing severe visual disorders and are within the main reasons for corneal grafting in Croatia and in the World in a past few years. We aim to find a link between these disorders and extra cellular matrix re-modellation molecules.

Methods

The activities of matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), pro-matrix metalloproteinase-13 (proMMP-13) and tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) were measured using an enzyme linked immunosorbent assay (ELISA) in three human corneal tissue layers (epithelium, stroma and endothelium) supernatants of the patients with keratoconus and bullous keratopathy which underwent the perforative keratoplasty procedure.

Results

MPP-2, MMP-9, proMMP-13 and TIMP-1 activity was detected in all samples. The epithelial layer showed significantly higher levels of MMP-9 and proMMP-13 in BK than in KC. Increased levels of MMP-2 ($p=0.07$) levels were found in bullous keratopathy compared to keratoconus patients. Epithelial TIMP-1 showed no significant difference in activity between KC and BK.

Conclusions

All these findings suggest an active degradation of the extra-cellular matrix in epithelial corneal layer in Bullous Keratopathy. No difference in the concentration of MMP-2, MMP-9, proMMP-13 and TIMP-1 between KC and BK in corneal stroma and endothelium suggest that neither of these molecules play important role in KC or BK pathogenesis, at least not in stroma and endothelium.

Amniotic membrane transplantation in the treatment of persistent epithelial defect on the corneal graft.

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Purpose

It has been shown that amniotic membrane transplantation (AMT) improves healing of the epithelium defects by preventing inflammatory cell infiltration and by reducing apoptosis in keratocytes. Epithelium of AM produces basic fibroblas growth factor, transforming growth factor-ß and intreleukin(IL)-1ra. Thick basement membrane serveces as an ideal substrate to suport growth of epithelial cells, since it facilitates migration of epithelial cells, reinforces adhesion of basal epithelial cells and promotes epithelial differentiation. These reactions explain why amniotic membrane can be used to facilitate epithelisation in persistent epithelial defects.Having in mind the healing properties of AM we investigated the efficacy of AMT in persistent epithelial defect (PED) on the corneal graft.

Methods

67 corneal grafts were prospectively followed up for presence of PED 10 months after surgery. PED was detected in 6 cases (8,9%) having surgery for: rejected graft (n=3), keratoconus on a second eye (n=2) and corneal perforation (n=1). Epithelial defect (ED) developed 14 +/- 7 days after surgery in four cases and 1,5 month in other two. Since ED was unresponsive to all previous treatments for more then 2 weeks, amniotic membrane (one or more layers) was placed on the corneal leasion, trimmed and sutured with resorptive continuous suture. Postoperatively eyes were bandaged with contact lens and steroid/artificial tears solutions were applied.

Results

Healing of the defect was obtained in 5/6 (83,3%) eyes. In one patient second AM transplantation was necessary. Mean epithelization time was 3 weeks (range 2-5 weeks). Four out of six patients retained the same BCVA while 2/6 patients improved their vision more than 2 lines. In 2 cases of deep PED several layers of AM were placed. Preoperative corneal thickness of 210 and 280 µm increased to 380 and 550 µm.

Conclusion

AM transplantation facilitates rapid healing of corneal epithelium. PED on the corneal graft unresponsive to conventional treatment can be effectively cured when covered with one or more amniotic membrane layers.

Influence of cryopreservation and air drying on amniotic membrane and impact of storage time on cryopreserved amniotic membrane

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Purpose

Cryopreserved amniotic membrane (Cryo-AM) is widely used in ocular surface surgery because of its positive effect on wound healing and its anti-inflammatory properties. A new peracetic acid/ethanol sterilized air-dried amniotic membrane (AD-AM) recently became available which might be an alternative to Cryo-AM. We compared AM preserved with both methods with regard to the release of wound-healing modulating proteins, the preservation of basement membrane components, and the ability to serve as a substrate for the cultivation of human limbal epithelial cells (HLECs). In addition to this we studied the impact of long-term storage on Cryo-AM.

Methods

Pieces of Cryo-AM and AD-AM from three different donors were incubated in serum free DMEM for five days at a ratio of 0.5 ml medium per cm^2 tissue. The culture supernatant was collected after an incubation period of 0.1, 24, 48, 72 and 120 h; in the case of AD-AM, this period was extended up to 14 days. For studying the influence of long term storage samples of Cryo-AM which were stored continuously at -80°C for an average storage time of 4, 15 and 24 months were also incubated in serum free medium. This medium was changed in 24 h intervals over a period of three days. The proteins TIMP-1, IL-1ra, CTGF and TGF-β1 were studied in the conditioned medium by means of Western blotting. Twenty human limbal epithelial cultures were initiated on both AD- and Cryo-AM. Limbal cultures on Cryo-AM and AD-AM were analyzed morphologically, and the outgrowth area was measured in 3-day intervals. Cryosections of all tissue samples were analyzed histochemically to detect the basement membrane components collagen IV, collagen VII, laminin, laminin 5 and fibronectin.

Results

The release of TIMP-1, IL-1ra and TGF-β1 from Cryo-AM was constant for the studied period. CTGF showed a stronger signal after 120 h. The proteins were detected in conditioned medium from all Cryo-AM samples independent from their storage time. None of the analyzed proteins, except for a small amount of IL-1ra, could be detected in the supernatant of AD-AM. An outgrowth of HLEC was observed in all cultures on Cryo-AM, but in only 30% of cultures on AD-AM. The outgrowth area on Cryo-AM was at all time points significantly higher than on AD-AM ($p < 0.0001$). Collagen IV, -VII, laminins and fibronectin were detectable in the basement membrane of all Cryo-AM samples, but only collagen IV and fibronectin were found in AD-AM.

Conclusions

Cryo-AM is a more suitable substrate for the cultivation of HLECs than AD-AM. The higher outgrowth rate of cultured limbal epithelium, releases of intact soluble wound-healing modulating factors and a better preservation of basement membrane components over long periods of storage suggest the superiority of Cryo-AM for use in ophthalmology in comparison to AD-AM.

SCIENTIFIC SESSION V

Tissue specific donor selection and processing

Keynote Lectures

Donor Selection for Lamellar Surgery

J Alvarez de Toledo MD

CENTRO DE OFTALMOLOGÍA BARRAQUER. BARCELONA

Corneal lamellar surgery has been reintroduced into the anterior segment surgical procedures in the last ten years due to the advances in corneal refractive surgery. The development of microqueratomes, femtosecond lasers, surgical instruments and the interest of the surgeons to treat the corneal diseases depending on the layers that are affected have made possible procedures like anterior and posterior lamellar grafting. Words like DALK, SALK, DSEK, DLEK, DSAEK are usually found in the majority of programmes of national and international cornea meetings.

Eye banks must be prepared to supply corneal tissue for these new procedures. Anterior lamellar keratoplasty does not need a donor with a healthy endothelium. Corneal tissue with optimal epithelium and stroma but with low endothelial cell count or endothelial diseases can then be accepted, stored and used for this purpose. On the other hand, for endothelial transplantation procedures such as DSAEK or DMEK, corneas with previous refractive surgery, dense arcus senilis or anterior non-viral opacities but with high endothelial counts can be accepted for storage and clinical use.

These new concepts oblige eye banks to acquire new examination instruments to carefully perform a complete evaluation of the donor cornea: slit lamp, keratometer or topographer, pachymeter and OCT will be routinely added to the eye bank armamentarium. Eye-bank technicians must also play a role in the preparation of the tissue. Microkeratomes to obtain DSAEK donors or surgical skills to prepare DMEK donor rolls are going to be incorporated into the technician routine work in the eye bank.

Surgeons must also be accustomed to receive tissue for the type of surgery they are going to perform instead of preparing it themselves. This has organizational implications for the eye bank-surgeons network that are going to be established in the near future. More tissue can then be accepted for grafting but surgeons must be aware of the kind of tissue they ask for.

The revival of lamellar procedures has new consequences for the eye bank and they must change and adopt new techniques and instruments to follow these surgical developments.

EURO-GTPs. Project funded by the European Union in the Framework of the Public Health Program

Esteve Trias, Jaime Tabera, Maria Zardoya, Jan Schoeter ,Arlinke Bokhorst, Audrey Laven, Deirdre Fehily, Wivine André, Shalaw Fawzi, Andrea Gareiss-Lok, Izabela Uhrynowska, Artur Kamiński, Jean Paul Pirnay,Gilbert Verbeken, Sari Sarkaniemi, Hanna Kankkonen, Katriina Aalto-Setälä, Daniela Vici, Ramadan Jashari, Nelleke Richters.

Objective

The project is being developed by 12 partners from 7 different European Countries

Project Coordinator; Transplant Services Foundation (TSF- Spain)
Partners ; BIS Foundation (Netherlands), Charite Universitatsmedizin (CBC-Germany) Centro Nazionale Trapianti (CNT-Italy), Banque Universitaire de Tissus (BUTB-UCL- Belgium), Hornhautbank München Gemeinnutzige (HBM-Germany), National Centre of Tissue and Cell Banking (KCBTiK-Poland), Hôpital Central de la Base Reine Astrid (HCB-QA-Belgium), Tampere Yliopisto. University of Tampere (Regea-Finland), Banca Tessuti della Regione Veneto (BTV-Italy), European Homograft Bank (EHB-Belgium), Euro Skin Bank (ESB- Netherlands).

The main objective of this project is to develop common Good Tissue Practices (GTPs) for European tissue establishments (TE), as well as a Training Model for TE personnel concerning the activities that are carried out in TE. These practices will increase the know-how and the level of performance of tissue banking staff, harmonizing the techniques used in order to provide tissues for transplant of high quality and safety, hence reducing the risk of disease transmission to recipients.

The aim will be achieved through developing:

- Generic Euro-GTPsthat will comprise practical instructions on (i) generic processes carried out in TEs, (ii) risk assessment and (iii) validation methods for donor screening and tissue procurement, processing, preservation and storage, as well as basic requirements concerning infrastructure, personnel, documentation management, etc.

- Tissue-specific Euro-GTPs according to each tissue type (ocular, cardiovascular, musculoskeletal, and skin). The specific GTPs will specify how to proceed during donor selection in correspondence to the type of tissue retrieved, and during the tissue recovery, processing, preservation and storage. Also, guidance on how to validate the different processes will be given. To asses the effectiveness of each tissue-specific guidelines, we will put them into practice in a tissue establishment with adequate environmental conditions. Every participant will asses the effectiveness of the GTP developed.

Rationale

The facts that enhance the need of the Euro-GTPs Guide are:

- There are not currently neither common procedures for how the procurement, the processing and the preservation of tissues for transplant should be carried out, nor for how the donor selection and screening of tissue donors according to the type of tissue retrieved should be done

- More and more TEs are being moved to fulfill the GMP requirements, which is being found to be difficult to meet because the own intrinsic properties of the tissues and cells

- The current regulation doesn't cover all the real activities that are being carried out in tissue and cells activities and leaves gaps which might decrease the quality and safety of tissues and cells

On going

There are several current working lines which will suppose a great support for this Euro-GTP project.

- Germany is developing good practices for specific tissues according to the German scenario

- France works in identifying gaps of the current regulation

- EUSTITE's project focus on optimising and harmonising the standards and methods applied in the inspection and accreditation of tissue procurement and tissue establishments within the EU.

EURO-GTP Guide will be a tool for TEs and inspectors that will let assure the quality and safety of tissues and cells through covering specific tissues features, solving gaps in the current regulations, establishing the minimum quality criteria for a tissue or cell freely circulation along EU, providing a more suitable and homogeneous regulatory reference framework for Advanced Therapies inspectors.

Oral Presentations

Does the improvement of cataract surgery techniques increase the viable corneas for transplant?

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Purpose

Criteria concerning cornea donors with previous cataract surgery has been a subject of discussion in the last years. Due to previous results with this type of donor, TSF halted recovery of phacoemulsified corneas from October 2003 to 2007. Because of the improvements in the surgical technique and improved instrumentation, these potential donors have once again been evaluated for corneal recovery. This study analyzes whether the source of viable corneas for transplantation from this group of donors has increased during the last years.

Methods

An observational, retrospective and comparative study was conducted. To that effect, complete corneal evaluation and specular microscopy endothelial cell count was performed. Data from the donor, retrieval and evaluation of corneas were registered in a database for analysis. This data was compared between two groups. Corneas of group A (n = 49) were obtained from January 2002 until December 2003 and corneas of group B (n=137) were obtained from January 2008 until July 2009.

Results

The percentage of corneal viability was 32.7 % for group A and 42.3 % for group B. In both groups, 19 % of the considered viable corneas were not used for transplantation. The average time that passed since cataract surgery till donation of corneas in group B was 4.77 ± 4.13 years. Endothelial cell count was clearly higher in group B (2002 ± 482 cells) than in group A (1797 ± 501 cells). Average endothelium cellularity was different in non viable corneas (1479± 433 cells/ mm^2 in group A vs. 1724± 479 cells/ mm^2 in group B). By contrast, viable corneas had similar endothelial cellularity (2230 ± 157 cells/ mm^2 in group A vs. 2280 ± 284 cells/ mm^2). Finally, the average donor age was slightly higher in group B (76.75 ± 6) than in group A (73.2 ± 5).

Conclusion

The viability of corneas from donors previously submitted of cataract surgery in the last years (group B) increased 10 % allowing to increase the number of retrieved corneas useful for transplantation. This result is probably explained by recent improvements of techniques, instrumentations and devices in cataract extraction.

Diagnosis of Photorefractive Keratectomy in Donated Whole Globes

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Purpose

To diagnose prior photorefractive keratectomy (PRK) in donated whole globes.

Methods

Donated whole globes with no previous history of refractive surgery on family interview were evaluated in the Central Eye Bank of Iran. After exposure to povidone iodine, cases with loose or defective epithelium were examined grossly under fluorescent light and then with slitlamp biomicroscopy and after photographing the suspicious cases to PRK, the excised corneas fixed in 10% formalin were processed for histopathological examinations.

Results

Among 6782 donated whole globes, 16 corneas from 8 donors were suspected to previous PRK based on gross observation of a disciform central to mid-peripheral non-transparent under fluorescent light, and the presence of a disciform superficial granular reflection from the same area on slitlamp biomicroscopy after gentle removal of a sector of the loose epithelium. Histopathological examination confirmed the preliminary diagnosis of previous PRK in all suspected cases.

Conclusion

Reevaluation of donor corneas after exposure to povidone iodine and gentle removal of a sector of abnormal-looking corneal epithelium in suspected cases is a safe and simple method for diagnosis of PRK with negative history.

Suture related complications after penetrating keratoplasty. Advocacy for posterior lamellar grafting?

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Background

Penetrating keratoplasty with mechanical trephines is currently standard treatment for corneal blindness. This operation requires graft suturing contrary to the emerging posterior lamellar procedures. Loosening or rupturing of graft sutures can give rise to microbial graft infections or graft rejections. We investigate herein the incidence of suture related graft infections, graft rejections and the percentage of resuturings in a large cohort after penetrating keratoplasty.

Patients and methods

We reviewed the follow up data of 2950 consecutive penetrating keratoplasties from the years between 1988 and 2003. We counted suture loosening, suture rupturing as well as consecutive microbial infections or graft rejections. All grafts hat been sutured with Hoffmann's double running cross stitch procedure.

Results

The percentage of suture loosening and suture rupturing totalled 5% after 3 years (Kaplan Meier estimation). Fourteen percent of this group experienced suture related graft infections. Graft rejections occurred more often as well (30% vs. 22% in the reminder without suture loosening, $p<0.01$).
A total of 8% required resuturings within the first three postoperative years. However, nearly half (4%) were performed for early postoperative leakage from the graft host interface. A second accumulation of resuturings occurred between the 12. and 18. months after surgery. These operations mostly became necessary after graft protrusion/ dehiscence following complete suture removal. Only 0.9% of the complete group suffered from microbial infections or graft rejections after suture loosening.

Conclusion

The overall percentage of severe sequela from suture loosening or rupturing is as low as 0.9%. This complication can thus be considered insignificant in comparison to e.g. graft rejections that occur in every fifths patient. The sutures should not be removed completely before the first 18 postoperative months in conventional penetrating keratoplasty. This policy will most likely reduce the percentage of resuturings for graft protrusion following complete suture removal.

Donor Cornea Preparation for Femtosecond Laser Assisted Keratoplasty

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Purpose

To describe the process of using the femtosecond laser and software to prepare donor corneal tissue for shaped penetrating keratoplasty (PK) and anterior lamellar keratoplasty (ALK) in an eye bank setting. The first 649 cases are reported here.

Methods

In a qualified environment, cold storage donor corneas that were suitable for transplant , were mounted on an artificial anterior chamber. Using the IntraLase Enable Keratoplasty™ software, the femtosecond laser was set to the surgeon requested parameters to make the desired shaped graft. The femtosecond laser procedure was then performed on the donor corneas. The technician finished the dissection of the corneal grafts with a blunt instrument and placed it in the preservation media. Subsequent slit lamp and specular evaluation occurred before ultimate distribution to the transplanting surgeons.

Results

649 donor corneas were prepared for transplant for multiple surgeons. 627 of the donor corneas were for PK and 22 corneas for ALK. The 627 PK corneas were cut in the following configurations: 546 ZigZag, 37 Mushroom, 22 TopHat, 22 Tongue/Groove, and 3 other. The average pre and post dissection endothelial cell densities were respectively 2996 and 2819 cells/mm2, exhibiting a 5.9% cell loss. The average donor age was 49.1 years old. No primary graft failure or intraocular infection were reported post surgery.

Conclusions

Eye bank prepared donor corneal tissue on the femtosecond laser is a safe and effective option for surgeons performing femtosecond laser assisted keratoplasty. This can be especially helpful to surgeons who may have challenges with the access and/or location of their femtosecond laser. Eye bank prepared tissue has the benefit of ensured quality by post-preparation evaluation and volume/experience of preparation.

Clinical results of femtosecond laser-assisted penetrating keratoplasty

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Purpose

Femtosecondlaser-assisted penetrating keratoplasty allows profiled trephinations as e.g. top hat and mushroom profiles.

Methods

In 2008 we performed 84 femtosecondlaser-assisted penetrating keratoplasties (53 tophat and 31 mushroom profiles). Mean follow-up currently is 8.2 months. We examined postoperative visual acuity, refraction, graft astigmatism (Orbscan topography), wound healing, time point of suture removal, occurrence of complications and of immune reactions.

Results

Mean visual acuity is 0.4 in the tophat group and 0.6 in the mushroom group (means were generated from logMAR values). Mean astigmatism is 6.2 diopters in both groups. Total suture removal was performed in 18 patients 9.5 months (average) postoperatively, 41 patients had one running suture left and 23 both running sutures. Eight immune reactions were observed.

Conclusions

Femtosecondlaser-assisted penetrating keratoplasty is a safe surgical method. Functional and refractive results seem to be worse than those of conventional trephination methods. However, early complete suture removal and therefore faster visual rehabilitation seems to be possible. Final evaluation of this surgical method is reasonable after complete suture removal in a larger cohort of patients.

Preparation and shipment of endothelial grafts by the eye bank: development and validation.

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Purpose

The preparation of lamellar endothelial grafts is a crucial step in the process of endothelial keratoplasty. Two 'schools' exist: assisted or manual lamellar dissection. Considering only the technical aspects of graft preparation, the former (using microkeratome or femtoseconde) is widespread but provides thicker grafts; the later provides thinner grafts but remains more confidential because it is difficult, less reproducible and requires a 'surgical' skill. To circumvent these difficulties, corneas could be pre cut in a specialized Eye Bank and sent in distant centers. Our aim was to assess the feasibility of this process using an original method able to measure the true endothelial cell asset of viable cells given to the surgeon.

Methods

Ten pairs of human corneas devoted to scientific use were organ cultured (OC) for 19±1.5 days before preparation. Endothelial cell density (ECD) was determined prior to the dissection, using a routine cell counting method with an image analysis system. One cornea, chosen at random, was dissected; the control cornea remained in OC medium. The dissection was performed by one surgeon (MM), on an artificial anterior chamber, endothelial side up, using a smooth spatula, on a 8mm diameter disc. The thickness of the lamellae could be chosen (either Descemet membrane alone or with a very thin stroma to ease the graft handling). In this study only thin posterior grafts were selected. Lamellae remained attached by a 10 degrees hinge. Pre cut corneas returned to their OC medium. Pairs of corneas were sent at controlled temperature to the distant center (640 km, from Rouen to St-Etienne) within 18-48 hours. A live/dead assay was then performed to compare the endothelial viability between both corneas. The triple labelling (Hoechst33342/Ethidium-homodimère/Calcein) coupled with image analysis on a research microscope, of the whole graft area, provided complementary quality criteria: raw ECD (H+), mortality rate (E+), area covered by viable cells (C+). A new notion of viable ECD was established: extrapolation corresponding to ECD that the corneas would have after redistribution of viable cells only.

Results

All endothelial lamellae remained adherent to the underlying stroma during transportation. Nevertheless the detachment was easy and atraumatic after section of the hinge. ECD in control and pre cut corneas were comparable before randomisation (respectively 2513±226 vs 2392±333 cells/mm^2). After dissection and shipment ECD were 2353±107 vs 1946±111 cells/mm^2 ($P<0.05$). Mortality rates were comparable, below 0,2%. Area covered with viable cells was higher in controls (90±1%) than in pre cut grafts (83±1%, $P<0.05$). The extrapolated 'viable ECD' were 2119±100 vs 1617±102 cells/mm^2 ($P<0,05$), corresponding to 119395±5726 vs 93882±5237 viable cells in 8 mm diameter grafts.

Conclusions

The triple labelling coupled with image analysis allows an extensive viability assessment of ECs. The new notion of viable ECD accurately represents the true endothelial resources of a cornea. This live dead assay can be used to assess EC viability after a large range of process or exposure to drugs. Although thin posterior graft pre cutting and shipment triggers a significant cell loss, such a process should be useful, provided that corneas with the highest ECD before cut are selected. The threshold of ECD for cornea selection nevertheless remains to be defined

More efficient use of donor corneas by using Descemet Membrane Endothelial Keratoplasty (DMEK)

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Purpose

To evaluate the potential reduction in discard rate of donor tissue by the introduction of DMEK.

Methods

Of 73 consecutive donor corneas, preparation error in harvesting Descemet grafts was studied, as well as the suitability of remaining anterior corneal tissue for deep anterior lamellar keratoplasty (DALK).

Results

Four grafts (5%) showed an inadvertent tear during stripping off Descemet membrane from the donor posterior stroma. Of the 73 donor corneas 18 (25%) showed anterior corneal abnormalities (*e.g.* arcus senilis <8.0 mm), so that the tissue was ineligible for DALK. Hence, (73 - 4 =) 69 DMEK and (73-18) 55 DALK procedures, *i.e.* a total of 124 transplantations could have been performed out of a pool of 73 donor corneas.

Conclusion

Although about 5% tissue loss may be anticipated in preparing DMEK grafts, the *overall* discard rate of donor tissue may be significantly reduced. Since a Descemet graft (for use in DMEK) as well as a full-stromal graft (for use in DALK) can often be harvested from just one donor cornea, the efficiency in the use of donor corneal tissue may increase to 170% (124/73).

Optical and confocal microscopic evaluation of endothelial donor rolls for DMEK

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Purpose

To study the histological characteristics of the endothelial monolayer after its manual dissection and preservation in organ culture media.

Material and methods

Eight human corneas not suitable for penetrating keratoplasty because of serology but with a healthy endothelium were evaluated with phase contrast microscopy with trypan blue and hypotonic sucrose solution. Endothelium with its Descemet´s membrane was manually dissected according to Melles technique in the eye-bank under sterile conditions. Descemet rolls were obtained successfully in all cases and stored in organ culture media at 32º C during 2 weeks. All the rolls were studied with phase contrast microscopy before and after the dissection, and with alizarine red staining and confocal microscopy after the culturing period.

Results

Endothelial monolayer presented signals of traumatic damage in the microscopic evaluation done immediately after the manual endothelial dissection. Main findings were disruption of the intercellular spaces and areas of non-response to hypotonic sucrose solution. After 2 weeks in culture, the intercellular swelling pattern returned to normality. No significant cell loss was observed during the culture. After culturing, the alizarine red staining showed small peripheral tears in one roll, and small clusters of focal damaged areas. Confocal microscopy corroborated these findings.

Conclusion

Manual preparation of Descemet rolls induces mild traumatic changes in the endothelial monolayer, mostly in the periphery of the donor. These changes are repaired during a period of organ culture. Due to these findings we suggest to use only corneas with high cell counts (>2400 cel/mm^2), perform a first evaluation of the endothelial status immediately after the dissection and store them for a 2-4 weeks period in organ culture, after which a second evaluation is mandatory before sending the tissue for a DMEK surgery.

Endothelial cell density after Descemet membrane endothelial keratoplasty (DMEK): 1-2 years follow-up

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Purpose

To evaluate donor endothelial cell density (ECD) following Descemet membrane endothelial keratoplasty (DMEK).

Methods

From a larger group of patients who underwent DMEK for Fuchs endothelial dystrophy or pseudophakic bullous keratopathy, complete ECD measurements were available of 43 patients with both 6 and 12 months follow-up, of which nine also had 2-years follow-up.

Results

For the group with 24 months follow-up, ECD averaged 2688 (+/- 235) cells/mm^2 before surgery, 1997 (+/- 593) cells/mm^2 at 6 months, 1841 (+/- 548) cells/mm^2 at 12 months, and 1608 (+/- 506) cells/mm^2 at 24 months after surgery. For the group with 12 months follow-up, ECD averaged 2629 (+/- 197) cells/mm^2 before surgery, 1855 (+/- 519) cells/mm^2 at 6 months, and 1688 (+/- 549) cells/mm^2 at 12 months after surgery. In both groups, the ECD decreased significantly between the preoperative and 6 month measurement ($p<0.05$).

Conclusion

Similar to earlier endothelial keratoplasty techniques, DMEK may be associated with a decrease in donor ECD of approximately 25% in the early postoperative phase.

Visual rehabilitation following Descemet membrane endothelial keratoplasty (DMEK)

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Purpose

To evaluate the visual rehabilitation after DMEK in the management of corneal endothelial disorders.

Methods

In 130 patients with Fuchs' endothelial dystrophy/bullous keratopathy DMEK was performed. A 3.0 mm clear corneal tunnel incision was made, the anterior chamber was filled with air and the Descemet membrane was stripped from the posterior stroma. A 9.5 mm DM roll was harvested from an organ cultured donor corneal-scleral rim and inserted into the recipient anterior chamber. The donor tissue was unfolded, positioned on the posterior stroma and secured by completely filling the anterior chamber with air for 45 min.

Results

In 18 eyes (14%), the primary graft did not function or was partially detached. These patients underwent Descemet stripping endothelial keratoplasty (DSEK) surgery successfully. In the remaining 112 DMEK eyes, 82% (92/112) reached a best corrected visual acuity of 0.5 or better, and 56% (63/112) reached 0.8 or better, at 3 months.

Conclusion

DMEK may offer quick and near complete visual rehabilitation in the management of corneal endothelial disorders.

Qualitative and quantitative parameters of pre-cut posterior corneal lamellae with stromal rim used for Descemet's Membrane Endothelial Keratoplasty with a Stromal rim (DMEK-S).

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Purpose

To prepare posterior corneal lamellae consisting of endothelium-Descemet's membrane (DM) with a stromal rim by manual technique in conditions of Ocular Tissue Bank Prague. To assess the endothelial quality of lamellae after the preparation and before performing the Descemet's Membrane Endothelial Keratoplasty with a Stromal rim (DMEK-S).

Methods

During the assessed period (10/08 - 10/09), 37 posterior corneal lamellae were manually prepared in Ocular Tissue Bank and sent for transplantation purposes. The live endothelial cells density (ECD-L) and percentage of dead cells (%DC) was assessed before, immediately after lamellar preparation and after 48 hours of storage under tissue culture at 31℃. Trypan blue 0.15% and sucrose 0.9% treatment were used before light microscopy evaluation of the lamellae.

Results

The mean ECD-L of corneas before the preparation was 2876 (2585-3333) cells/mm^2, the mean %DC was 0.02. Immediately after the lamellar preparation, we found 1.45 % of DC in average and the mean ECD-L decreased to 2835 (2478-3268) cells/mm^2. After 48 hours of storage under tissue culture, the mean ECD-L was 2739 (2391-3081) cells/mm^2 and the mean %DC was 1.07.

Conclusions

A technique not necessitating microkeratome or laser devices for the preparation of endothelium-DM lamellae with stromal rim in conditions of an eye bank was evaluated. Corneal lamellae with ECD-L higher than 2300 cells/mm^2 are sent for DMEK-S.

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SCIENTIFIC SESSION VI

Workshop session

Keynote Lectures

“New” Infectious Diseases in the U.S. & Donation of Human Tissues and Cells

Scott A. Brubaker, CTBS

Chief Policy Officer, American Association of Tissue Banks (AATB)

Regional changes in climate that facilitate insect vector migration, ease of global travel by many, and the ability of novel diseases to cross species and further develop, are some ingredients in the recipe for the emergence of infectious diseases. These “new” diseases reaching a nation or region can affect the safety of tissue and cell transplantation. Determining relevance of a disease should be based on science that we seek, as eye and tissue banking professionals, to ultimately support decisions made regarding donor screening requirements. Are all new diseases true threats to the transplantation of tissues? The provision of safe tissue for recipients in need is everyone’s goal and science should support practice, which includes establishing donor eligibility criteria and donor testing that makes sense.

Since early 2008, the US Food and Drug Administration (FDA) issued two draft guidance documents that propose requirements for testing human donors of tissue for new relevant diseases. One proposal includes a requirement to test all donors for West Nile Virus using a nucleic acid test methodology (NAT) and the other to test all donors for antibodies to Trypanosoma cruzi, the parasite that causes Chagas’ Disease. Importance of these diseases to organ transplantation and blood transfusion has been established but relevance to the transplantation of various tissues is not understood. Controlled studies need to be performed and are being developed by the AATB and the Centers for Disease Control and Prevention (CDC) with input from the FDA and the Eye Bank Association of America (EBAA). Workshops are planned and scientific study protocols are being designed.

Testing tissue donors for antibodies to human T-lymphotropic virus type I and type II (anti-HTLV-I and anti-HTLV-II) has evolved and is relevant only to tissue types that are rich in viable leukocytes. H1N1 influenza, arboviruses like Dengue, and a newly recognized enterovirus, XMRV, are in the news and can affect donor screening.

Reacting to new diseases using the ‘precautionary principle’ philosophy can substantially increase health care costs and reduce the availability of safe tissue for transplant. Proper risk assessment based on science and facts is preferable, which means tissue banking professionals must communicate and work closely with regulators to establish sound requirements. Implementing vigilance and surveillance programs regarding tissue recipient adverse reactions can play an important role in supporting decision-making so this must also evolve.

Implementing the Regulation of Banking in the EU

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The European Union tissues and cells Directives provided the framework to Member States to build systems for the regulation of tissue and cell procurement and banking. The Directives obliged Member States to nominate 'Competent Authority(ies)' with responsibility for the implementation of a number of fundamental regulatory elements: inspection, authorization, data collection and publication and adverse event and reaction reporting and management. Many Member States previously had no infrastructure for this type of tissue and cell regulation. Competent Authorities and inspectorates with widely varying experience and expertise were nominated across the EU; some are pharmaceutical inspectorates, some are public health inspectorates, some are national transplant organizations and some are organisations newly established specifically for tissue and cell regulation. A need to harmonise the approach to implementation if the Directives was recognised and a three-year project with this primary objective was co-funded by the European Commission under the Public Health Programme.

The EUSTITE project (European Union Standards and Training in the Inspection of Tissue Establishments) brought together European regulators of tissues and cells to collaborate in the development of common approaches to the implementation of many of the key elements of the European Directives on Tissues and Cells. The project partnership included 11 organisations, 10 of which are Competent Authorities for tissues and cells in 10 Member States, and the World Health Organisation. It was led by the Italian National Transplant Centre. Representatives of Competent Authorities in 26 of the 27 Member States participated in project events such as workshops, training courses, conferences and a pilot programme on vigilance of serious adverse events and reactions. Project activities and outputs have been widely disseminated by presentations at conferences in Europe and beyond and by a number of publications.

The project gathered and disseminated detailed information regarding the existing systems for inspection and vigilance and provided opportunities for the evaluation of their strengths and weaknesses and for achieving consensus on best practice, as well as tools to support its delivery. Workshops and conferences in Dublin, Madrid and Rome explored key issues in inspection and vigilance with experts from within and beyond the EU. A questionnaire survey and a series of 9 exchange inspection visits facilitated the definition of guiding principles which were then incorporated in practical guidance documents.

Two of the key outputs of the project were the EUSTITE Inspection Guidelines and the Tools and Guidance for Vigilance and Surveillance, both of which can be found on the project website (www.eustite.org). The Guidelines have now been used by the European Commission as the basis for a draft Decision on guidelines concerning inspections and control measures in the field of tissues and cells of human origin and an associated Operational Manual for Competent Authorities on the Inspection of Tissue and Cell Procurement and Tissue Establishments. These Commission documents are in the final stages of approval. The Tools and Guidance for Vigilance and Surveillance has been the subject of a pilot programme involving 22 Competent Authorities from 20 Member States. The document includes criteria for the reporting of Serious Adverse Events (SAE), scales for the assessment of Severity and Imputability of Serious Adverse Reactions (SAR) and an Impact Assessment Tool to support Tissue Establishments and Competent Authorities in deciding the most appropriate response to individual SAR and SAE. The SAE reporting criteria and the severity and imputability scales were incorporated into the guidance provided by the European Commission to Member States to support the completion of their annual SAR/E report to the Commission in 2009.

Starting in the Summer of 2008, the project ran a series of 4 training courses for tissue and cell inspectors in the EU, as part of a work-package led by the Austrian partner. Each course consisted of a 7 week e-learning module followed by a 3 day residential module. The demand for participation was high and, in total, 71 inspectors from 26 Member States participated. The residential courses, held in Vienna, Sofia, Copenhagen and Como, were interactive case study

based courses. They provided ample opportunity for inspectors from widely varying backgrounds and organisations to interact with each other and to exchange ideas and information.

As the project closed, with a Final Conference in Warsaw on December 2nd to 4th, the project partners and collaborators were looking to the future. A new project proposal focusing on Vigilance and Surveillance issues has been submitted to the European Commission and funding has been agreed in principle. Contract negotiation for this project is underway and it is hoped that it will follow in the footsteps of EUSTITE by delivering further practical and helpful tools to EU regulators and practitioners in the field.

POSTERS

Bacteriological and fungal contaminations of corneal organ cultures media in french eye banks: results 2008

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Purpose

In France, the law 98-535 of July 1st 1998 established rules for the use of tissues and put the French Health Products Agency (Afssaps) in charge of the evaluation, inspection and control of these products. The Laboratories and Controls Department (DLC) is in charge of external quality control of tissues. In this field, since 2005, the DLC has registered all the micro-organisms found in the bacterial or fungal contamination of corneal organ cultures media in French Eye Banks. An analyse of this data and a comparison of different types of contaminations between 2005 and 2008 has been performed.

Methods

Each year, every Eye bank sends their list of all the micro-organisms found in their corneal organ cultures media analysed. The step in the process when the contamination appeared, the microbiological tests used, the germ time detection and the total number of controlled corneas are all specified.

Results

In 2008, 19/20 Eye Banks participated in the inventory. 8,3% (621/7493) of French cornea were destroyed because of the bacterial or fungal contamination. 74% of Eye Banks have a cornea contamination rate below 10%. The number of controls, the procurement site number and the size activity of the banks don't affect the level contamination. During the storage process, the majority of Eye Banks performed 3 microbiological controls. The contaminations were detected by blood culture bottle methods (80% of banks) and 80% used a specific fungi media. The distribution of the germs was: 78% of bacteria (26,2% genus *Staphylococcus*), 15% of yeasts (85,7% genus *Candida*), 2% of fungi (non-identified, *Fusarium*…), 0,3% of mycobacterium, 3% of mixture (bacteria, yeast and bacteria, yeast and fungi…), 2% of germs was not identified. Sampling media are more contaminated than the other media.
Since 2005, the national contamination rate has significantly decreased to 10,6% to 8,3% in 2008 ($p<0,001$). The germ distribution was equivalent to the value of 2005.

Conclusions

This data allows us to better know in a more exhaustive way the micro-organisms responsible of the cornea contaminations. This inventory enables us to define the germs which will be sent to the banks within the new collaborative studies lead by the DLC with an aim of validating or updating bacteriological reference frames. The contamination levels of the different Eye banks have decreased between 2005 and 2008. Two corrective actions were performed: mobilization of staff to assure a safe procurement in order to lower the rate of contamination and to identify germs to know the origin of contamination. In addition, since 2005, the contaminations with *Sphingomonas paucimobilis* have been identified like coming from the bain-maries at the time of mediums defrosting. It thus seems urgent to take measures making it possible to decrease these contaminations rates responsible for the destruction of approximately 300 corneas since 2005.

The assessment of pathogenic prions in the brains of eye tissue donors: two years' experience in the Czech Republic

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Purpose

The aim of this study was to assess the presence of pathogenic prions in the brain tissue of eye donors and to evaluate the benefits of two years obligatory testing in the Czech Republic.

Methods

Brain tissue was retrieved during autopsies of eye donors of three tissue banks in the Czech Republic. The frozen specimens obtained from the frontal lobe were transported to the Czech National Reference Laboratory for the Diagnosis of Human Prion Disorders. The presence of pathogenic prions was tested using the Prionics®-Check WESTERN kit. Confirmative western blotting using one of two different clones of monoclonal anti-PrP antibody was performed as well.

Results

No pathogenic prions were found in any of the 1142 tested specimens. One specimen revealed weak positivity at initial screening; however, repeated examination of the specimen and other specimens from different locations in the brain of the same donor did not confirm the presence of pathogenic prions. The negative result was confirmed by the National CJD Surveillance Unit, University of Edinburgh, UK.

Conclusions

The absence of pathogenic prions from all of the 1142 tested specimens corresponds to the presumed very low risk of transmission of Creutzfeldt-Jakob disease through corneal graft transplantation. Because of the rarity of this disorder, a larger series of tested samples should be evaluated to obtain statistically significant findings. Although such testing increases the safety of donor eye tissue, it increases also the expense, causes organizational difficulties and may extend the time needed to release the tissue for grafting.

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External quality control of cornea organ culture media: results 2007-2008

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Purpose

In application of the articles L5311-1 and L5311-2 du CSP, Afssaps is in charge of the tissue control in the Laboratories and controls department. A pilot study with all the French Eye bank was led from February to May 2005 and allowed us to validate the microbiological control feasibility and to initiate the routine control. From January 2007 to December 2008, 6 tour controls were carried out.

Methods

112 packages were transported to Afssaps at a range temperature between +2°C and +10°C from 20 Eye Banks. 84% of the boxes contained a temperature sensor. Altogether, 459 corneal media (33% of sampling media, 65% of storage media and 2% of deswelling media) were analysed from a microbiological standpoint and sample quality related to transport conditions in parallel by Eye Banks and compared with Afssaps results.
80% of laboratories use aerobic and anaerobic blood cultures methods manual or automated and 20% seeded conventional methods.
80% of laboratories use a specific fungi media. The techniques used by Afssaps were Blood culture bottles and Sabouraud medium. The types of Bact/Alert seeded media depend on volumes of received media.

Results

In 95% of the cases, the temperature of the samples was in conformity at the reception and 9% of the 89 temperature curves analyzed during transport did not conform. The majority of the samples (325/459) were analyzed at Afssaps between 0 and 5 days after the banks' analysis. 3% of the results (13/459) were not in conformity on the microbiological level and 2% of the results (8/459) were unmatched between Afssaps and the corneas banks. 7 samples out of 8 were positive in Afssaps and negative in the banks and 1 sample out of 8 were negative in Afssaps and positive in the banks.

Conclusion

97% of the controlled media were negative on the bacteriological and fungal level. Positive controls found in Afssaps and negative in the banks allowed: either to destroy the corneas intended for the graft, or to set up a biovigilance alert when the cornea had been grafted and to carry out a follow-up of the receiver. This control is only effective if the banks send their media and that we carry out our control nearly at the same time as they do theirs, so when discordance appear the corneas can be destroyed before graft.
Since October 2005, positive controls number decreased from 5% in 2005-2006 to 3% in 2007-2008. This reduction follows the same tendency, like the bacteriological and fungal contamination rate of the corneas declared positive annually by the French eye banks (10,4% in 2005-2006 and 8,6% in 2007-2008). This difference between the contamination controls rate and the annual declarations is mainly due to the storage media for which it exists a factor of 2 between the rate found in controls and in the annual declarations. A media drawing lot sent to Afssaps for control would be probably necessary. Moreover results of these quality controls in the long term will enable us to standardize the methods in order to work out reference frames of control.

Revitalizing effects in transplant cells of donor cornea *in vitro* under the influence of homologous cellular peptides

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Purpose:

To study the protective and revitalizing effects of tissue-specific regulatory peptides on corneal graft cells in the course of preservation by means of organ culture with the purpose to enhance viability of endothelial cells after posterior lamellar keratoplasty.

Methods:

The preparation NeyDIL №37 St III (Cornea) of the vitOrgan Arzneimittel GmbH was used. The preparation contains regulatory peptides from fetal and juvenile animal cornea in concentration 100 mkg/ml. Organ cultures of corneal graft were incubated at 31℃ in the medium of Eye Bank of The Fyodorov Eye Microsurgery Federal State Institution. Ten corneas of 10 donors were included in a control group, and 10 corneas of the same donors were included in an experimental group. Every day the organic preparation NeyDIL № 37 was added into the experimental medium (the concentration was 1 mkg/ml). The incubation was 14 days. The endothelial density was counted up in a routine way before the incubation and 3, 6, 10 and 14 days later. Further, the method of scanning and transmission electronic microscopy was used.

Results:

During 14 days of organ culture the loss of endothelial corneal cells in the control group was 4.2%, in the experimental group it was 2.7% (similar to physiological loss in vivo). The electron microscopy of endothelial cells in the control group, showed the increased hydration of cells and the destruction of mitochondrial membranes. In the experimental group these changes were insignificant.

Conclusions:

The pronounced revitalizing effect (the modulation of compensatory-adaptive mechanisms) of tissue-specific regulatory corneal peptides (preparation NeyDIL № 37) on water-salt and energy metabolisms of endothelial corneal cells, mitotic activity in stroma of corneal graft has been revealed during the conservation in organ culture.

Effect of Traumatic Hyphema on Endothelial Cell Indices of Donated Corneas

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Purpose

To compare the endothelial cells of donated corneas with hyphema after non-penetrating trauma with those of control group.

Methods

51 corneas from 44 donors with traumatic hyphema and 113 corneas from 68 donors with cardiovascular cause of death were enrolled. Specular microscopy indices in each group were statically compared by using generalized estimation equation (GEE) models and were adjusted for age.

Results

No significant difference in the endothelial cell indices including co-efficient of variation (P value = 0.253), cell density (P value = 0.191), mean cell area (P value = 0.233) and percentage of hexagonal cells (P value = 0.198) was noted between the two groups.

Conclusion

It seems that hyphema has no significant adverse effect on endothelial cell indices in the donated corneas.

Penetrating keratoplasty and “iris-claw“ lens - is it safe for endothelium?

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Purpose

The loss of the corneal endothelial cells has been observed in patients who underwent penetrating keratoplasty (PK). The implantation of new generation of „iris claw“ phakic IOL has been shown to cause insignificant endothelial cell loss. In our prospective case series we observed the endothelial cell loss in patients that either underwent PK and implantation of PCIOL or PK and implantation of “iris claw“ lens (Verisyse).

Methods

Prospective case series. In the first group of 9 patients scheduled for PK, implantation of Verisyse was performed due to the absence of the posterior capsule support. 2 of these patients had angle supported ACIOL, 4 patients were aphakic and 3 had posttraumatic cataract with ruptured posterior capsule. The second group of 12 patients had standard „triple“ procedure (PK + PCIOL). BCVA of both groups of patients prior the operation were hand movement in 12 patients, light perception in 7 patients and 0,05 in 3 patients. The preoperative endothelial cell count of the donor graft was obtained from the eye bank and was 2400±400 cells/mm^2 average. The follow up was 6-10 months.

Results

Six months after the operation „Verysise“ patients maintained transparent graft and BCVA ≥ 0.5 except one patient who developed glaucoma. The endothelial cell count and morphology were estimated on the specular microscope (CSO,Firenze, Italy) on a monthly basis. Endothelial cells loss in patients with PK and Verysise was 25 % and in patients with „triple“ procedure was 20% in the observation period, respectively.

Conclusion

There was no significant difference in the endothelial cell loss between the group of patients who had PK and Verysise as compared to those with implanted PCIOL.

Fatal post-transplant disorders of donor origin following liver and kidneys transplantations

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Purpose

To present three cases of fatal post-transplant disorders of donor origin following liver and kidneys transplantations.

Methods

On 13th June 2005 a 19 -year-old patient was admitted to the Department of General and Transplantation Surgery with brain stem death after sudden circulatory arrest. The patient was previously healthy with no complaints on his general health condition. On 17th June a death of the patient was certified and he was qualified as an organ donor. Nine hours later the liver, both kidneys, the heart, and both corneas were received.

Results

The intrasurgical histopathological examination of a thymus biopsy revealed a thymoma. The liver recipient was a 31-year-old man, kidneys recipients: a 59-year-old woman and a 32-year-old man; corneas recipients: a 62-year-old and 45-year-old men. The heart was not qualified for heart valves homographs. Few months later liver and two kidneys recipients developed acute lymphoblastic leukemia and T-cell lymphoma, respectively. Routine histopathological examination of donor`s thymus revealed a precursor T-cell lymphoblastic lymphoma of thymus. Despite intensive treatment all these patients died in 2006. The postoperative course of two corneal recipients was uneventful, both patients are till now in good general condition.

Conclusions

1. Despite a progress in organ and tissue transplantation, transmission of donor`s disorders to transplant recipients can occur.
2. The case of our donor is, to our knowledge, the first reported multiple transmission of donor`s disease to liver and kidneys recipients.

Trichophyton fungal keratitis

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Purpose

To report on severe consequences of Trichophyton fungal keratitis infection in a contact lens wearer.Fungal keratitis represents one of the most difficult forms of microbial keratitis. Difficulties arise in making the correct diagnosis, recognizing the disease by its clinical characteristics, and obtaining confirmation from the microbiology laboratory. Since there is often a delay in making correct diagnosis, the infection is often more advanced when correct treatment is started. Moreover, topical antifungal preparations are not as advanced as antibiotics for bacterial infections, and are hard to obtain. Fungi can cause severe stromal necrosis and enter the anterior chamber by penetrating an intact Descemets membrane. Once in the anterior chamber, the infection is very difficult to control, in part due to poor penetration of antimycotic agents. The most common pathogens are filamentous fungi (Aspergillus and Fusarium spp.) and Candida albicans. The incidence of Trichophyton is 5%.

Methods

Case report.

Results

A 22 years old female (used to wear contact lenses) developed corneal melting syndrome, spontaneous perforation of the cornea and complicated cataract of the left eye as a complication of a keratitis. Patient was admitted in such condition from another hospital after 2 weeks of unseccsesful keratitis treatment with topical and systemic antibiotics. At the beginning of the diseases only conjunctival swab was taken, which was sterile. After admitance, a piece of diseased corneal tissue and the sample from the anterior chamber were urgently taken. Lavage of the anterior chamber with cefuroxim and vancomicyn was performed. As soon as donor tissue was available (48 hours after admission), urgent keratoplasty(PK) was performed together with extracapsular cataract extraction and the implantation of the intraocular lens in the posterior chamber. Vitreal body was clear at that time. The patient was treated with diflucan and ciprofloxacin (systemic therapy) and chlorhexidine, brolene, levofloxacin, polimyxin B, and dexamethason/neomycin (topically). Cytology evaluation was performed following excisional biopsy of the intracameral portion of the lesion.The presence of Trichophyton sp. was confirmed. Itraconazole and garamycin were included in the systemic therapy, as suggested by our Infectious Diseases Department. Two weeks after the operation, microorganisms invaded the vitreus and caused endophthalmitis. Pars plana vitrectomy and the lavage of the anterior chamber with vancomycin was performed. Corneal graft was clear for 17 days and decompesated 28 days after the PK. Despite repeated PPV to reduce the amount of microorganisms, patient lost light sensation and developed phthisis. Evisceration and the implantation of silicon prothesis was finally done.

Conclusion

Trichophyton may cause a severe disease of both the anterior and posterior part of the eye. Urgent penetranting keratoplasty is a method of choise in treating corneal perforation caused by infectious keratitis, but without the eradication of microorganisms it can not restore the vision or save the eye. Prompt diagnosis and treatment of Trichophyton keratitis are essential to save patient's sight.CONCLUSION: Trichophyton may cause a severe disease of both the anterior and posterior part of the eye. Urgent penetranting keratoplasty is a method of choise in treating corneal perforation caused by infectious keratitis, but without the eradication of microorganisms it can not restore the vision or save the eye. Prompt diagnosis and treatment of Trichophyton keratitis are essential to save patient's sight.

How improve the corneal quality?

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Retrospective analysis of the results of controls on 2036 corneas stored in our Cornea Bank will give a better knowledge of factors influencing quality of corneas for transplantation especially in terms of endothelial cell number. So, these data will help us improve the selection of the donors to increase the number of grafted corneas.

Purpose

Retrospective study reviews the effects of donor age, refrigeration and time between death and procurement on corneal quality controls, including serologies, endothelial density and risk of contamination.

Methods

2036 corneas were received in Lyon (France) cornea bank from the 1st October 2006 to 30 September 2009.

To study the effect of donor age, corneas have been divided in 6 groups according to donor age (from 0 to 59 years old donors, 60-69Y, 70-79Y, 80-89Y and more than 90 years)
To study the effect of the time between death and procurement, corneas have been divided into 6 groups: < 4 hours, 4 to 8 hours, 8 to 12 hours, 12 to 16 hours, 16 to 20 hours and 20 to 24 hours.
To study the effect of refrigeration, corneas have been divided in two groups, collected from refrigerated or non-refrigerated donors.
Presently the rate of grafted corneas of each group has been compared to the global rate. Then « Laboratoire de Mathématiques Expérimentales » of Marseille is doing a multivariate analysis that we will present.

Results

Waiting for the multivariate statistical analysis, the results show clearly that among the 2036 corneas received in our bank, 46% (935) were grafted, 33,4% (680) were discarded for due to low endothelial density, 16,4% (334) for serological exclusion, 3,0% (62) for contamination and 1,0% (21) for clinical selection exclusions. For this last cause of elimination, the low rate is due to the fact that selection is already done before harvest.

The results show the influence of:

Refrigeration because 49.4% of corneas provided by non-refrigerated donors instead of 42.3% of those provided by refrigerated ones are validated for transplantation.

Interval between death and procurement and donor age and because (The graft rate increased from 46% (global) to 51.5% when the time interval between death and cornea procurement was <12h and from 46% (global) to 54% when the donor was <70 years old).

Conclusions

All these 3 factors (refrigeration, donor age, time between death and procurement,) influence quality of cornea. The multivariate analysis in course will give us more information.