We would like to take this opportunity to thank these organisations for their kind support and sponsorship of this event

Contents

Programme Summary

08.30 - 09.00: Registration

09.00 - 09.05: Chairman's Welcome and opening remarks

09.05 – 10.15: Research Presentations
Chairs Dr A Hopkinson and Prof HS Dua

10.20 – 10.40: Ocular aberrations: measurement, correction, and function
Dr EAH Mallen, Bradford University

10.40 – 11.00: Coffee Break (Poster and Trade Exhibitions)

11.00 – 12.05: Research Presentations
Chairs Dr E Stewart and Mr WM Amoaku

12.10 – 12.30: Clinical impressions of Keratoconus
Dr KH Weed, Dundee University

12.30 – 13.30: Lunch Break (Poster and Trade Exhibitions)

13.30 – 14.15: The Norman Galloway Lecture
Descemet Membrane Endothelial Keratoplasty, the thinner, the better
Professor Friedrich Kruse, Erlangen, Germany

Symposium: Added Value Intraocular Implants – Premium IOLs
Chairman Prof HS Dua

14.15 – 14.35: Toric implants
Mr Michael Lavin, Manchester

14.35 – 14.55: Multifocal implants
Mr Paul Rosen, Oxford

14.55 – 15.15: Accommodative lens implants
Mr Milind Pande, Hull

15.15 – 15.35: Tea Break (Poster and Trade Exhibitions)

15.35-15.55: Phakic implants
Mr Bruce Allan, London

15.55 – 16.10: Aniridia lenses
Professor Harminder Dua, Nottingham

16.10 – 16.25: Quality of vision with premium IOLs
Jean-Jacques Gicquel, Poitiers France

16.25 - 16.35: Discussion

16.35-16.45: Awarding of Research Presentation and Poster Prizes

16.45 -17.00: Chairman's concluding remarks and close of meeting

History of the Norman Galloway Lecture

The Norman Galloway Lecture was endowed in 1996, by Mr Nicholas R Galloway, Consultant Ophthalmologist at the University Hospital Queen's Medical Centre Nottingham (retired 2001), in memory of his father. This has since become a key feature of what is now a nationally recognised symposium.

Norman Patrick Galloway was born at Rhynie in Aberdeenshire on 27th March 1895 and died in Rempstone near Loughborough, Leicestershire on 2nd February 1976. He was a graduate of the University of Edinburgh and became a House Physician in the Edinburgh Royal Infirmary. During the First World War he served with the Army in South Africa, afterwards deciding to take up Ophthalmology. He obtained his DOMS in Oxford and during his time in Oxford met his future wife Eileen Thompson, the daughter of a general practitioner in Nottingham.

In 1922 he was appointed Clinical Assistant to the Nottingham and Midland Eye Infirmary and five years later, in 1927, he was elected Honorary Surgeon. He held this appointment through World War II and, in 1948, with the advent of the National Health Service, became Consultant Ophthalmologist. In the 1920's, Norman Galloway was an active member of the British Medical Association and helped to organise the meeting that was held in Nottingham in 1926. At a national level, for many years he supported the Midland Ophthalmological Society, regularly presenting papers, and in 1951 was appointed their President. He was also a member of the Council of the Oxford Ophthalmological Congress. He saw the introduction of antibiotics and steroids and, during the difficult post-war period, helped to steer the Hospital House Committee through the numerous negotiations involved with the formation of the National Health Service. He was also instrumental in gaining funding for the Eye Hospital extension to the wards and outpatient department. From 1950 to 1951 he was President of the Nottingham Medico-Chirurgical Society.

During his working life, Norman Galloway saw and helped to implement great changes in the practice of Ophthalmology in Nottingham. The old outpatient system where the doctor stood by a desk facing a queue of patients was replaced by consulting rooms and the building of the new extension allowed the introduction of special clinics. Nottingham had an Ophthalmic Nursing School before the war and at an early stage had an Orthoptic Department. Norman Galloway retired from the hospital in March 1959 after 34 years of service. His patients remember him as a kindly man who preferred one-to-one relationships. He tended to avoid public speaking whenever possible.

Nicholas R Galloway

Previous Norman Galloway Lectures

2010 **East and West** Professor D Wong, University of Liverpool and Hong Kong

2009 **The Good, the Bad and the Ugly: The Metastatic Potential of Uveal Melanoma** Prof IG Rennie, Sheffield

2008 **Paediatric Ophthalmology – where next?** Professor A Fielder, London

2007 **Paraneoplastic Retinopathies** Professor J-J De Laey, Ghent, Belgium

2006 **When topical steroids fail: Managing severe anterior segment inflammation** Mr JKG Dart, Moorfields Eye Hospital, London

2005 **Wavefront-guided keratrorefractive surgery: Advantages and limitations** Professor D Azar, Massachusetts Eye Infirmary, Harvard University, Boston, USA

2004 **Normal Tension Glaucoma** Professor R Hitchings, Moorfields Eye Hospital, London

2003 **Exploring the topographic and inner world of the cornea to the horizon of the iris plane: contemporary imaging of the anterior segment of the eye** Professor CNJ McGhee, University of Auckland, NZ

2002 **Prospects of treating inherited retinal diseases** Professor AC Bird, Institute of Ophthalmology, University College London

2001 **Classification and Treatment of Posterior Uveitis** Professor JV Forrester, University of Aberdeen

2000 **Herpes Simplex Viral Keratitis : What HEDS (Herpetic Eye Disease Studies) has taught us** Professor PR Laibson, Wills Eye Hospital, Philadelphia, USA

1999 **Management of Traumatic Ptosis** Mr JRO Collin, Moorfields Eye Hospital, London

1998 **Stargardt's Macular Degeneration** Professor LA Donoso, Wills Eye Hospital, Philadelphia, USA

1997 **Diabetic Retinopathy – a tolerable disease** Professor DB Archer, Queen's University, Belfast

Honorary Delegates

Nomination of delegates as "Honorary delegates" of the Symposium was considered for the first time in 2006. This was to recognise individuals who had supported the meeting and contributed to it over the years. These delegates have the privilege of full participation and attendance in the meeting as guests of the Symposium.

Mr Nicholas R Galloway, Nottingham (2006)

Professor Larry Donoso, Wills Eye Hospital, Philadelphia (2010)

Mr A A Zaidi, Rotherham, UK (2011)

Programme

9.00 am **Welcome and Introduction**
Professor HS Dua, Conference Chairman

9.05 am **Research presentations:**
Chairs: Dr A Hopkinson and Prof. HS Dua

9.05 am Donor site complications in auto limbal and living related allo limbal transplantation
Miri, A., Said, D. and Dua, H.S. (University of Nottingham)

9.12 am The functional significance of foveal abnormalities in albinism measured using spectral-domain optical coherence tomography
Mohammad, S., Kumar, A., Thomas, M., Degg, C., Sheth, V., Gottlob, I., Proudlock, F.A. (University of Leicester)

9.19 am Investigation of the Relationship between Differentiation of Limbal Epithelial Cells and Their Released Soluble Factors
Wright, B., Mi, S., Chen, B. and Connon, C.J. (University of Reading)

9.26 am Characterisation of Corneal Stromal Cells as a Novel Mesenchymal Stem Cell Source
Dhillon, P., Branch, M., Hashmani, K., Hopkinson, A. And Dua, H.S. (University of Nottingham)

9.33 am Location and Structure of Amniotic Membrane Affects the Success of Limbal Stem Cell Expansion
Connon, C., Chen, B. (University of Reading)

9.40 am Corneal stromal stem cells
Hashmani, K., Hopkinson, A., Branch, M. and Dua, H.S. (University of Nottingham)

9.47 am Antimicrobial peptide expression by ocular surface cells in response to Acanthamoeba infection: *in vitro* and *ex vivo* studies
Otri, A.M., Mohammed, I., Abedin, A., Cao, Z., Chen, P., Hopkinson, A., Panjwani, N. and Dua, H.S. (University of Nottingham)

9.54 am Corneal Intra-epithelial Neoplasia: In Vivo Confocal Microscopic Study with Histopathological Correlation
Alomar, T.S., Nubile, M., Lowe, J. and Dua, H.S. (University of Nottingham)

10.01 am Psoriasin (S100A7) expression in human corneal epithelial cells
Chen, P., Hopkinson, A. and Dua H.S. (University of Nottingham)

10.08 am Superficial Keratectomy, a solution option for Salzman's Nodular Degeneration
Issa, M., Leyland, M. (Oxford Eye Hospital)

10.20 am **Guest Lecture:**
Ocular aberrations: measurement, correction, and function
Dr EAH Mallen, Bradford University

10.40 am to 11.00 am ***Refreshments; Poster Exhibition and Trade Exhibition***

11.00 am **Research Presentations *(continued):***
Chairs: Dr E Stewart and Mr WM Amoaku

11.00 am Adherent ocular bandage for clear corneal incisions used in cataract surgery
Calladine, D., Ward, M., Packard, R. (King Edward VII Hospital, Windsor)

11.07 am High resolution *in-vivo* imaging in achromaptosia
Thomas, M.G., Kumar, A.S., Kohl, S., Proudlock, F.A., Gottlob, I. (University of Leicester)

11.14 am Intraocular Pressure Elevation and Glaucoma Escalation after Deep Anterior Lamellar Keratoplasty
Musa, F. (University Hospitals Nottingham)

11.21 am Corneal Nerve Aberrations in Bullous Keratopathy
Al-Aqaba, M.A., Alomar, T., Lowe, J., Dua, H.S. (University of Nottingham)

11.28 am Cross-linking of Amniotic Membrane for Clinical Use
Clare, G., Allen, C., Hopkinson, A. and Dua, H.S. (University of Nottingham)

11.35 am Correlation of Central and Peripheral Corneal Thickness in Healthy Corneas
Fares, U., Otri, A.M., Al-Aqaba, M.A., Dua, H.S. (University of Nottingham)

11.42 am De-novo Malignancies after Tacrolimus therapy in the management of ocular inflammatory diseases
Bhatt, U., Raj, D., Dua, H.S. (University Hospitals Nottingham)

11.49 pm Efficacy and side-effects of hypertonic sodium chloride ointment (5%) in the treatment of recurrent corneal erosions
Tsatsos, M., Savant, V., Prydal, J. (Leicester Royal Infirmary)

12.10 pm **Guest Lecture:**
Clinical impressions of Keratoconus
Dr KH Weed, Dundee University

12.30 pm to 1.30 pm **Buffet lunch; Poster Exhibition and Trade Exhibition**

1.30 pm **Introduction to the Norman Galloway Lecture**
Professor HS Dua

1.35 pm **The 15th Norman Galloway Lecture**
Descemet Membrane Endothelial Keratoplasty, the thinner, the better
Professor Friedrich Kruse, Erlangen, Germany

Symposium on Added Value Intraocular Implants – Premium IOLs
Chairman Prof HS Dua

2.15 pm **Toric implants**
Mr Michael Lavin, Manchester

2.35 pm **Multifocal implants**
Mr Paul Rosen, Oxford

2.55 pm **Accommodative lens implants**
Mr Milind Pande, Hull

3.15 pm to 3.35 pm ***Refreshments; Poster Exhibition and Trade Exhibition***

3.35 pm	**Phakic implants** *Mr Bruce Allan, London*
3.55 pm	**Aniridia lenses** *Professor Harminder Dua, Nottingham*
4.10pm	**Quality of vision with premium IOLs** *Jean-Jacques Gicquel, Poitiers, France*
4.25pm	**Discussion**
4.35 pm	**Research Presentation and Poster Prizes**
4.45 pm	Chairman's concluding remarks and discussion
5.00 pm	**CLOSE We hope you enjoyed the meeting**

This meeting has been award 6 CPD points by the Royal College of Ophthalmologists, if you require a certificate of attendance this can be collected at the registration desk along with your payment receipt

Please turn in your name badge for recycling

See you next year, 27th January 2012!

Previous Prize Winners

Nottingham Research Trophy
A rolling trophy and an individual shield awarded to the best presentation in the clinical research category considered by a panel of judges on the day.

2010 Architecture and Distribution of Human Corneal Nerves
M Al-Aqaba, Nottingham

2009 Voluntary modulation of involuntary eye movements during reading
M G Thomas, Leicester

2008 Glaucoma in an elderly Caucasian population (The Bridlington Eye Assessment Project)
A Bhan-Bhargava, Nottingham

2007 Digital infrared pupillometry for comparing cocaine with apraclonidine testing when investigating Horner's syndrome
A Shwe-Tin

2006 Linear regression modeling of rim area to discriminate between normal and glaucomatous optic nerve heads: The Bridlington eye assessment project.
M J Hawker

2005 Can patching be improved in amblyopia treatment?
M Awan, Leicester

2004 Amniotic membrane transplantation for ocular surface reconstruction: A seven year retrospective analysis.
V S Maharajan, Nottingham

2003 Effect and compliance of strabismic amblyopia monitored with the occlusion dose monitor
M Awan, Leicester

2002 A prospective, case controlled study of the natural history of diabetic retinopathy and maculopathy after uncomplicated phacoemulsification cataract surgery in patients with Type 2 diabetes.
D Squirrell

2001 The detection of T-Cell activation by retinal autoantigen in uveitis patients using cytokine flow cytometry
J Morgan, Nottingham

2000 Spatial localisation in esotropia - is extraocular muscle proprioception involved?
C Weir

1999 A method to visualise leukocytes in the retinal and choroidal circulation in vivo
P Hossain

1998 Tacrolimus in high-risk corneal and limbal transplants
C M Sloper, Nottingham

1997 Rapid suture management of post-keratoplasty astigmatism
A R Sarhan, Nottingham

David Meyer Research Trophy
A rolling plaque and an individual shield awarded to the best presentation in the basic science research category considered by a panel of judges on the day.

2010 High resolution spatial and temporal expression profile of FRMD7 in neuronal tissue provides clues for pathogenesis and treatment
M G Thomas, Leicester

2009 Interleukin-1 beta induced RNase-7 expression requires MAPK but not NF-kB signalling
I Mohammed, Nottingham

2008 Human Choroidal Endothelial Cell Growth Factor signalling in Age-Related Macular Degeneration
E A Stewart, Nottingham

2007 Mutations in FRMD7, a Novel Gene, Cause X-linked Congenital Idiopathic Nystagmus
S Thomas, Leicester

2006 Amniotic membrane for ocular surface reconstruction: Donor variations and handling affect membrane constituents
A Hopkinson, Nottingham

2005 In vivo confocal microscopy: Corneal changes following retinal detachment surgery with intra-ocular silicone oil
K H Weed

2004 The isolation and characterisation of adult human sub-macular inner choroidal endothelial cells
A Browning, Nottingham

2003 Characterisation of an in vitro model for studies into age related macular degeneration
R D Hamilton, Nottingham

Nottingham Poster Prize
An individual shield awarded to the best poster presentation considered by a panel of judges on the day.

2010 Human defensin 9, a 'functional' host defence protein
I Mohammed, Nottingham

2009 Expression Pattern of Anti-microbial peptides (AMPs) in Acanthamoeba Keratitis
A M Otri, Nottingham

2008 Malignancies after Tacrolimus Therapy in the management of ocular inflammatory disease
M Mathew, Nottingham

2007 A 24-months follow-up of severe ocular burns with impression cytology
J-J Gicquel, Poitiers, France

2006 Retinal features in children with Down's syndrome
P Ji

2005 Intravitreal triamcinolone acetonide in the management of refractory uveitis
H Kolli

2004 National survey of management of acquired nystagmus
I Choudhari, Leicester

2003 Interactive teaching in ophthalmology
P Tesha, Leicester

2002 The taut thickened posterior hyaloid (TTPH)
D Thomas

2001 Hyaluronic acid promotes the migration of corneal epithelial cells in vitro
R Amankwah, Nottingham

2000 Quantitative assessment of cytokine mRNA and secreted protein in proliferative vitreoretinopathy
I A El-Ghrably, Nottingham

1999 Does ethnic origin influence the incidence or severity of keratoconus?
A Pearson

1998 Modified Sheridan Gardiner vision test with semi-transparent card
R Ahmed, Nottingham

1997 Stem cell deficiency of the corneosleral limbus : a new approach to surgical management
D Raj, Nottingham

Research Presentation Abstracts

In order of presentation on the day

Donor Site Complications in Auto Limbal and Living Related Allo Limbal Transplantation

Miri, A.[1,2], Said, D.[1,3] and Dua, H.S.[1]

[1]University of Nottingham, England, UK, [2]Department of Ophthalmology, Aleppo University, Aleppo, Syria and [3]Research Institute of Ophthalmology, Cairo, Egypt.

Purpose:

To study the long term changes at donor sites and safety implications for donor eyes used for harvesting tissue for autologous and living related donor limbal transplants.

Methods:

50 donor sites of limbal tissue belonging to 25 healthy eyes (23 human subjects) were examined. The corneas and limbus of donor eyes were assessed for symptoms and visual acuity and examined by slit-lamp biomicroscopy and in vivo confocal microscopy (IVCM) with particular emphasis on the donor sites and central cornea.

Results:

Mean follow up was 41 ± 38 months. All eyes had symptoms of ocular discomfort up to 4 weeks postoperatively and remained asymptomatic thereafter. No patient reported subjective reduction in visual acuity. Mean best corrected visual acuity (LogMar fraction) preoperatively was 0.076 ± 0.19 and postoperatively was 0.09 ± 0.17 (p value = 0.57).

Observed complications were filamentary keratitis and sub-conjunctival haemorrhage in four eyes. IVCM confirmed that the central corneal epithelium remained normal in all eyes. The re-epithelialised donor site was covered with conjunctival epithelium in 17 sites of 10 eyes and with corneal epithelium in 7 sites of 5 eyes.

Conclusions:

Limbal donation of two clock hours of the superior and inferior limbus with 3 x 3 millimetres of adjacent conjunctiva was a safe procedure in this group of patients, demonstrating stable vision and an intact corneal epithelium during the follow up period. Donor sites can be re-epithelised by multiple layers of either corneal or conjunctival epithelium and is associated with deep stromal scarring.

The Functional Significance of Foveal Abnormalities in Albinism Measured using Spectral-domain Optical Coherence Tomography

Mohammad, S.[1], Kumar, A.[1], Thomas, M.[1], Degg, C.[2], Sheth, V.[1], Gottlob, I.[1], Proudlock, F.A.[1]

[1]University of Leicester, Ophthalmology group, Robert Kilpatrick Clinical Sciences Building, Leicester Royal Infirmary, Leicester, UK
[2] Medical Physics Department, Leicester Royal Infirmary, Leicester, UK

Purpose:

The relationship between foveal abnormalities in albinism and best corrected visual acuity (BCVA) is unclear. We used high resolution spectral-domain optical coherence tomography (SD-OCT) to assess the functional significance of foveal morphology in patients with albinism.

Methods:

7x7x2mm volumetric scans of the fovea were acquired using high resolution SD-OCT (3µm axial resolution) in 47 patients with albinism. The B-scan nearest to the centre of the fovea was identified using signs of foveal development. The thickness of each retinal layer at the fovea and foveal pit depth was quantified manually using ImageJ software and compared to best corrected visual acuity (BCVA).

Results:

Total photoreceptor layer thickness at the fovea was highly correlated to BCVA (p=0.0008, r=-0.501). Of the photoreceptor layers, outer segment length (OSL) was most strongly correlated to BCVA (p<0.0001, r=-0.641). In contrast, there was no significant correlation between either total retinal thickness or pit depth and BCVA (p>0.05). This was due to an inverse correlation between total photoreceptor layer thickness and total processing layer thickness (p<<0.0001, r=-0.696).

Conclusion:

Neither the total retinal thickness nor the pit depth are reliable indicators of visual deficit as patients with similar overall retinal thickness had widely varying foveal morphology. In albinism we find the size of the photoreceptor outer segment to be the strongest predictor of BCVA. Our results suggest that detailed SD-OCT images of photoreceptor anatomy provided a useful tool in assessing the visual potential in patients with albinism.

Investigation of the Relationship between Differentiation of Limbal Epithelial Cells and Their Released Soluble Factors

Wright, B., Mi, S., Chen, B. and Connon, C.J.

Stem Cells and Nanomaterials Laboratory, Reading School of Pharmacy, University of Reading, RG6 6UB, Berkshire, United Kingdom

Purpose:

Limbal stem cell deficiency (LSCD) is a leading cause of corneal blindness. We investigate mechanisms underpinning limbal stem cell differentiation to understand LSCD therapy.

Methods:

Limbal epithelial cells (bovine model) were cultured in serum-free medium (1, 2 and 3 days). The presence of stem or stem-like cells was determined by the colony forming efficiency (CFE) assay. Cell and culture medium proteins separated using sodium dodecyl sulphate polyacrylamide gel electrophoresis (12 µg total protein) were visualised by silver staining. Differentiation (cytokeratin 3 (CK3)) and undifferentiation (cytokeratin 14 (CK14)) were assessed by immunoblotting.

Results:

Cell colonies formed on a fibroblast feeder layer (CFE), demonstrating the presence of stem or stem-like cells amongst initially isolated cells. CK14 increased at day 2 and decreased at day 3, whilst CK3 was maintained at greater levels than CK14 at day 3. Changes in CK3 and CK14 levels provided further evidence for the presence of limbal stem or stem-like cells. Distinct profiles of 8-20 kDa proteins within conditioned medium and cells were observed. Proteins from conditioned medium are potentially growth factor-like products secreted from cells. Changes in CK3 and CK14 levels correlated with variations in levels of 8-20 kDa proteins within conditioned medium. Greater levels of proteins (8-20 kDa) in conditioned medium were observed after 2 and 3 days than 1 day. A corresponding increase in levels of cytokeratin 14 was also observed after 2 days.

Conclusions:

These data suggest mechanisms of limbal epithelial cell differentiation potentially involve secretion of low molecular weight (8-20 kDa) proteins.

Characterisation of Corneal Stromal Cells as a Novel Mesenchymal Stem Cell Source

Dhillon, P., Branch, M., Hashmani, K., Hopkinson, A. And Dua, H.S.

Division of Ophthalmology and Visual Sciences, University of Nottingham

Background:

Multipotent mesenchymal stromal cells (MSC) are found in connective tissue of many organs in the body. These fibroblastic cells display stem cell (SC) differentiation and proliferation properties and are currently used for tissue engineering in a clinical setting. Corneal stromal cells (CSC) are also fibroblastic in morphology but have not been fully characterised although their SC potential has been alluded to in several publications.

Hypothesis:

CSC are MSC.

Methods:

CSC harvested from the limbal region of human donors were cultured *in vitro* and characterised using set criteria defined by the International Society of Cellular Therapy (ISCT) to identify MSC. A profile of positive and negative MSC cell-surface markers were analysed using flow cytometry at passage 3 and the cells were then differentiated into adipocytes, osteoblasts, chondroblasts and analysed using standard staining techniques. Finally, the CSC were clonally expanded to assess their proliferative capacity and uniformity. First trimester foetal liver MSC were used as a control throughout the experiment.

Results:

The CSC were spindle-shaped, plastic adherent, and were positive for CD29, CD90, CD105, CD44 and were negative for CD11b, CD19, CD34, CD45 and HLA class II antigen. The cells showed differentiation potential into adipocytes, osteoblasts, chondroblasts and were able to be clonally expanded.

Conclusion:

This work is the first to demonstrate CSC fulfil the criteria for MSC characterisation. Therefore in the fullness of time CSC and MSC may be exploited therapeutically for corneal regeneration.

Location and Structure of Amniotic Membrane Affects the Success of Limbal Stem Cell Expansion

Connon, C., Chen, B.

Stem Cells and Nanomaterials Laboratory, Reading School of Pharmacy, University of Reading, RG6 6UB, Berkshire, United Kingdom

Purpose:

Amniotic Membrane (AM) is commonly used as a substrate for limbal stem cell expansion. Previously we have shown structural variations in different parts of AM (Connon et al, 2010). Here we describe the effect of these differences on the subsequent expansion of limbal stem cells.

Methods:

AM samples were collected from areas adjacent to the placental disc (proximal AM) and approximately 10cm from placental disc (distal AM) (n>10) then washed and frozen prior to use.

Bovine limbal epithelial cells were expanded upon the AM for 15 days in basal medium.

Expression of differentiation markers and basement membrane proteins were compared by immunohistochemistry (IHC) between limbal epithelial cells cultured on proximal and distal AM (n=6). Scanning electron microscopy was used to examine the proximal and distal AM surface (n=3).

Results:

Limbal epithelial cells cultured on intact proximal AM showed well stratified (layer number>6) and undifferentiated cells whereas on distal AM cells were poorly stratified (layer number <3) and highly differentiated. SEM showed a rough surface on the distal region, but in proximal AM, epithelium showed a healthy condition. No difference was found in Collagen IV however Laminin expression in proximal AM was strong and sustained, whereas in the distal region, it was slightly weaker and transient.

Conclusions:

Our findings are the first to explore the effect of structural variations between proximal and distal AM on limbal stem cell expansion, and we highlighted that the differences within AM can alter cell micro-environment and change the phenotype of cultured cells.

Corneal Stromal Stem Cells

Hashmani, K., Hopkinson, A., Branch, M. and Dua, H.S.

University of Nottingham, Division of Ophthalmology and Visual Sciences

Purpose:

To characterise the stem cell properties of corneal stromal cells (CSC) and to investigate their potential for differentiation into corneal epithelial cells (CEC).

Methods:

CSC from donor rims were cultured for up to 9 passages and assessed against stem cell criteria. Investigations included flow cytometry to evaluate the expression of mesenchymal (MSC) and haematopoietic (HSC) markers. Furthermore, the ability of CSC to differentiate into adipocytes, osteoblasts and chondroblasts was assessed by histochemistry and qPCR.

CSC were expanded in 2 different culture media. The cells from both cultures were sorted into 2 separate populations based on HSC marker expression. These 4 populations were assessed for MSC characteristics. The potential of each of these sub-groups to differentiate into CEC was analyzed by flow cytometry and qPCR.

Results:

CSC showed both HSC and MSC marker expression up to 4 generations. The different culture media showed clear distinction in the marker profile and differentiation potential of CSC.

CSC separated by CD34 expression showed a significant difference in their expression of SC markers and their ability to differentiate. The differently cultured cells all produced CEC. However, one population showed a clearly superior differentiation capacity.

Conclusion:

A population of CSC may have stem cell properties. Stimulation of these cells may be used to generate CEC. This has potential for clinical treatment of ocular surface disease.

Antimicrobial Peptides Expression by Ocular Surface Cells in Response to Acanthamoeba Infection: *In vitro* and *Ex vivo* Studies

Otri, A.M.[1], Mohammed, I.[1], Abedin, A.[1], Cao, Z.[2], Chen, P.[1], Hopkinson, A.[1], Panjwani, N.[2] and Dua, H.S.[1]

[1]University of Nottingham, Division of Ophthalmology and Visual Sciences
[2]New England Eye Centre, Tufts University School of Medicine, Boston, Massachusetts, USA

Aims:

To study the *ex vivo* gene level of human beta defensins (hBD) 3 and 9 in healthy controls and patients with *Acanthamoeba* keratitis (AK) during acute stage and after healing, then highlight the *in vitro* gene expression of all the ocular AMPs in human corneal limbal epithelial cells (HCLE) stimulated with *Acanthamoeba castellanii* (AC)

Methods:

The *ex vivo* study: Impression cytology technique was used to obtain ocular surface cells from 8 healthy controls, 7 patients with acute AK and 5 after complete healing. HBD3 and hBD9 gene expression were analyzed using real time polymerase chain reaction (QPCR).

The *in vitro* study: Human corneal limbal epithelial cells were exposed to AC at different time points, up to 9 h, the genomic profile of the AMPs was analysed at these time points by QPCR. Corneal limbal epithelial cells not infected with AC were used as controls.

Results:

The *ex vivo* study demonstrated the significant downregulation of hBD9 and upregulation of hBD3 during the active AK, whereas the *in vitro* gene profile showed that seven of the eight studied AMPs were significantly upregulated. Human beta Defensin 3 (hBD3) showed a significant 10-fold upregulation and Ribonuclease-7 (RNase-7) showed an early and consistent increase in the exposed cells. Human beta Defensin 1 (hBD1) was the only downregulated AMP.

Conclusions:

This study highlighted the AMP-mediated ocular defence in vision-threatening AK. Using AMPs singly or in combination is a promising avenue for further exploration in the treatment of the sight threatening *Acanthamoeba* keratitis.

Corneal Intra-epithelial Neoplasia: In Vivo Confocal Microscopic Study with Histopathological Correlation

Alomar, T.S.[1], Nubile, M.[2], Lowe, J.[3] and Dua, H.S.[1]

[1]Division of Ophthalmology and Visual Sciences, School of Clinical Sciences, University of Nottingham, United Kingdom.
[2]Ophthalmology Clinic, University "G d'Annunzio" of Chieti-Pescara, Italy.
[3]Division of Histopathology, School of Molecular Medical Sciences, university of Nottingham, Nottingham, United Kingdom.

Purpose:
To ascertain *in vivo* confocal microscopic features of corneal/conjunctival Intraepithelial Neoplasia (CIN) and correlate these with histology of the same lesions. A diagnostic technology has been evaluated as well.

Methods:

Four cases of unilateral CIN (three men and one woman) were examined with the Heidelberg Retina Tomogram II (HRTII) Rostock Cornea Module (RCM) confocal microscope before, during and after treatment. Corneal epithelial samples were taken by alcohol delamination technique in two cases and impression cytology (IC) in the other two. Morphometric analysis of confocal and histological images was carried out and the findings correlated. Four controls (all men, 2 with limbal stem cell deficiency, one with a limbal lesion and one with diffuse keratoconjunctival proliferation) were similarly examined. Two of these had biopsy for histological examination. Main outcome measure comprised the degree of correlation between histology and confocal microscopic features of CIN.

Results:

Dysplastic cellular changes were noticed on histopathology and correlated well with confocal microscopy, in different corneal epithelial layers. Bright nucleoli within huge nuclei and ill-defined cell borders were a feature of the basal epithelium on histopathology and confocal microscopy. Sub-basal corneal nerves were not visualised on confocal microscopy in areas affected by CIN. These features disappeared in response to treatment cycles as the basal epithelium reverted to its normal pattern as seen by confocal microscopy.

Conclusion:

Confocal microscopy findings highly correlate with histological features in CIN. Confocal microscopic features of CIN as defined in this study will enable a reliable diagnosis in a non-invasive manner. Confocal microscopy will also allow real time monitoring of the condition during treatment.

Psoriasin (S100A7) Expression in Human Corneal Epithelial Cells

Chen, P., Hopkinson, A. and Dua H.S.

Division of Ophthalmology and Visual Sciences, University of Nottingham

Introduction:

Psoriasin (S100A7) is involved in a proinflammatory axis associated with various inflammatory diseases, implying its participation in the innate immunity system. Meanwhile, there is an increasing awareness that the coordinated interactions between various receptor molecules in the mammalian innate immune system, such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs), lead to a cooperative response being mounted against a range of pathogens. We would like to explore the expression of psoriasin in human corneal epithelial cells triggered by TLRs and NLRs.

Methods:

HCLE cell line was treated with TLRs and NLRs specific agonists. Gene expression of psoriasin was measured by quantitative real-time PCR, flow cytometry and immunocytochemistry were performed to detect the cytoplasmic protein level in response to stimuli.

Results:

Gene expression of psoriasin was significantly induced by bacterial lipoprotein, peptidoglycan and muramyl dipeptide in a time-dependent manner, and a much stronger induction has been shown when co-stimulated with ligands above. In contrast, the cytoplasmic protein level showed a notable up-regulation at the early stage of stimuli.

Conclusion:

Bacterial lipoprotein, peptidoglycan and muramyl dipeptide would be treated as the principle inducers for psoriasin. In addition, co-stimulation with these inducers has shown the synergistic effects under the tight control by pro- and anti-inflammatory responses. The time course of psoriasin gene expression seems to indicate an indirect mechanism of regulation involving pathogens mediated cytokines release and further induction of this gene.

Superficial Keratectomy: a Solution Option for Salzman's Nodular Degeneration

Issa, M., Leyland, M.

Oxford Eye Hospital

Purpose:

To review the nature and treatment options for Salzman's Nodular Degeneration with focus on Superficial Keratectomy.

Method & Results:

Didactic review of treatment options for Salzman's Nodular Degeneration with a video demonstration of Superficial Keratectomy as an option in certain circumstances.

Conclusion:

Salzman's Nodular Degeneration is an uncommon condition but can cause significant discomfort and can become disabling. We review the nature of this condition and review treatment options.

Adherent Ocular Bandage for Clear Corneal Incisions Used in Cataract Surgery

Calladine, D., Ward, M., Packard, R.

Prince Charles Eye Unit, King Edward VII Hospital, Windsor, UK.

Purpose:

To assess an adherent ocular bandage for clear corneal incisions (CCIs) in cataract surgery using optical coherence tomography (OCT).

Methods:

Patients having coaxial microincision cataract surgery (MICS) were allocated to an adherent ocular bandage group or to a control group. The CCIs were examined postoperatively within 2 hours and at 24 hours and 7 days using OCT imaging and a slitlamp fluorescein 2% Seidel test.

Results:

The ocular bandage group comprised 22 eyes and the control group, 23 eyes. The mean intraocular pressure (IOP) in the immediate postoperative period was significantly lower in the control group (13.4 mm Hg G 5.28 [SD]; range 5 to 23 mm Hg) than in the bandage group (19.4 G 5.94 mm Hg, range 11 to 29 mm Hg) ($P<.001$, t test). In the bandage group, all incisions were Seidel negative. In the control group, 1 main incision was Seidel positive. In 2 cases, the bandage successfully captured a micro-leak and thus maintained an intact anterior chamber.

Differences in OCT architectural features between the bandage group and control group were noted.

Conclusions:

The adherent ocular bandage protected the incisions, selectively adhering to deepithelialized areas and rapidly clearing from reepithelialized areas. The bandage helped maintain a more desirable IOP in the immediate postoperative period, likely by preventing micro-leaks.

High Resolution *In-Vivo* Imaging in Achromaptosia

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[2] Molecular Genetics Laboratory, Institute for Ophthalmic Research, Department for Ophthalmology, University Tuebingen, Germany

Purpose:

To characterize the retinal changes in patients with achromatopsia using an ultrahigh resolution spectral optical coherence tomography (UHR-OCT)

Methods:

UHR-OCT (Copernicus, 3µm axial resolution) was used to obtain scans of 30 eyes from 15 patients with achromatopsia and 40 eyes from 20 control subjects of similar ages without any ocular pathology. A 3-D scan program (743 x 75; A scan x B scan) sampling a 7x7mm retinal area centered at the fovea was used to obtain tomograms of the fovea. Individual B-scans at the fovea were exported and analyzed using ImageJ for reflectance profiles and morphological abnormalities.

Results:

A characteristic "punched out" hyporeflective zone (HRZ) was noted in 8/15 patients; this was an age dependent phenomenon ($p = 0.001$). The area of the HRZ had an asymmetric distribution with the nasal area being significantly greater than the temporal area ($p = 0.002$). In all patients there was disruption of the IS/OS junction at the foveal and/or parafoveal regions. 6/15 patients also had a disrupted COST reflectivity. There was significant ($p = 1.1 \times 10\text{-}6$) ONL thinning in the achromats compared to controls which was an age-dependent phenomenon ($p = 0.0002$). Foveal maldevelopment was seen in 10/15 patients. The achromats also had a significantly reduced foveal depth ($p = 7.7 \times 10\text{-}6$) and RT ($p = 1.46 \times 10\text{-}9$) compared to controls.

Conclusion:

We describe a range of signs in achromatopsia that can be detected using UHR-OCT. IS/OS and COST reflectivity disruption, presence of HRZ and ONL thinning are signs of cone photoreceptor degeneration. The latter two are age-dependent phenomenon which suggests that achromatopsia is a progressive disorder. In addition we describe foveal maldevelopment which represents mainly fetal developmental defect linked to cone photoreceptor degeneration.

Intraocular Pressure Elevation and Glaucoma Escalation after Deep Anterior Lamellar Keratoplasty

Musa, F.

Nottingham University Hospitals

Objective:

To determine the prevalence and severity of glaucoma after deep anterior lamellar keratoplasty (DALK) and its effect on visual function.

Design:

Retrospective cohort study.

Participants:

71 eyes of 59 patients.

Methods:

Case notes review of patients undergoing DALK procedures at Leeds University Hospitals between the 1st of January 2000 and the 31st of December 2005.

Main Outcome Measures:

Glaucoma incidence or escalation, graft survival, visual function.

Results:

Data was collected for 71 eyes of 59 patients. The mean period of follow-up was 53.3 months (median 60; range 1 to 95 months). The initial diagnosis was keratoconus in 57 cases (80%); central corneal scarring in 5 cases (7%); herpes simplex keratitis in 4 cases (6%); lattice dystrophy in 4 cases (6%); and 1 case of corneal dermoid. Temporarily raised IOP thought to be related to topical steroid use occurred in 12 (17%) cases. Sustained IOP rise occurred in 3 cases. None of these patients had progressive disc changes or visual field defects suggestive of glaucoma and all had well controlled IOP on topical, single drug therapy.

Conclusions:

The long term risk of glaucoma following DALK using the Melles manual dissection technique is low. Ocular hypertension after DALK is infrequent and can be controlled on topical medication alone.

Corneal Nerve Aberrations in Bullous Keratopathy

Al-Aqaba, M.A.[1], Alomar, T.[1], Lowe, J.[2], Dua, H.S.[1]

[1]Division of Ophthalmology and Visual Sciences, School of Clinical Sciences and [2] School of Molecular Medical Sciences, University of Nottingham Medical School, Nottingham, UK.

Purpose:

To study the corneal nerves in patients with chronic bullous keratopathy.

Design:

Prospective observational case series with histological evaluation.

Methods:

We studied 25 eyes of 25 bullous keratopathy patients of different aetiologies (17 females, 8 males; mean age, 76.3 years) as well as 6 eyes of 6 normal control subjects (5 males, one female; mean age, 38 years). All subjects were scanned by laser scanning confocal microscope. Five corneal buttons obtained following penetrating keratoplasty from 5 of the above patients and 6 normal control corneal buttons were stained as whole mounts with acetylcholinesterase (AchE) method for corneal nerve demonstration and scanned in multiple layers with NanoZoomer Digital Pathology scanning microscope.

Results:

The density, branching pattern and diameter of sub-basal nerves were significantly lower in corneas with bullous keratopathy compared with normal corneas (density: 4.42±1.91 mm/mm^2 vs. 20.05± 4.24 mm/mm^2; branching pattern: 36.02±26.57% vs. 70.79±10.53%; diameter: 3.07±0.64 µm vs. 4.57± 1.12 µm). Aberrations such as localised thickenings or excrescences, abnormal twisting, coiling and looping of the (mid) stromal nerves were observed in the study group both by *in vivo* confocal microscopy and on histology.

Conclusions:

Striking alterations in corneal innervation are present in corneas with bullous keratopathy which are unrelated to any specific aetiology of bullous keratopathy. This study provides histological confirmation of novel *in vivo* confocal microscopy findings related to corneal nerves in bullous keratopathy.

Cross-linking of Amniotic Membrane for Clinical Use

Clare, G., Allen, C., Hopkinson, A. and Dua, H.S.

Division of Ophthalmology & Visual Sciences, University of Nottingham

Purpose:

The clinical use of amniotic membrane (AM) in ocular surface surgery is hampered by enzymatic degradation of the collagenous biomaterial. We attempted to strengthen the membrane by cross-linking the collagen scaffold.

Methods:

We used a combination of immersion of the AM in 0.1% riboflavin A solution for 30 minutes, followed by exposure to ultraviolet A (UVA, 365 nm) for 30 minutes. The protocol was adapted from clinical techniques to treat keratoconus. The type I and III AM collagen fibres were purified by salt precipitation and examined by gel electrophoresis to investigate the results of the attempted cross-linking. Bands were identified by mass spectroscopy. Transmission electron microscopy (TEM) was performed on cross-linked and control AM, and interfibrillary distances were measured. Biomaterial testing was carried out to investigate the tensile characteristics of the cross-linked membrane. Enzyme digestion of AM with bacterial collagenase and matrix metalloproteases -1 and -2 was investigated by gel electrophoresis. Finally, ninhydrin assays were performed to determine the cross-linking index.

Results:

We showed that type I and III collagen bands disappeared from gel electrophoresis following cross-linking, suggesting that the AM fibrillar collagens had become polymerised. However, TEM interfibrillary distance measurements, enzyme digestion assays and biomaterials tests were inconclusive. Ninhydrin assays, however, proved that no substantial cross-linking was present.

Conclusions:

Cross-linking of AM by riboflavin/UVA irradiation most likely causes scission of collagen molecules and does not strengthen the membrane or increase resistance against enzymatic digestion. Chemical cross-linking methods may be more effective.

Correlation of Central and Peripheral Corneal Thickness in Healthy Corneas

Fares, U., Otri, A.M., Al-Aqaba, M.A., Dua, H.S.

University of Nottingham, Division of Ophthalmology and Visual Sciences

Introduction/Purpose:

To study the thickness profile of the normal cornea in order to establish any correlation between central and peripheral points.

Methods:

Sixty seven eyes of 40 patients were subjected to central corneal thickness measurement (CCT) with an ultrasound pachymeter (UP) and corneal thickness mapping with the Oculus Pentacam. The corneal apex thickness (CAT), pupil centre thickness (recorded as CCT and corresponded to CCT of UP) and thickness at the thinnest location (CTL) were obtained and compared with each other. Corneal thickness data at 3mm and 7mm temporally, nasally, superiorly and inferiorly from the corneal apex were obtained. The mean corneal thickness values along the 2,4,6,8 and 10 millimetres diameter concentric circles, with the CTL as the centre, were also obtained. The above data at different points were statistically correlated by Pearson product-moment correlation coefficient.

Results:

There was no significant difference between CCT readings measured by UP and Pentacam *(p=0.721)*. There was high positive correlation between the CAT values and the thickness at 3mm ($R \geq 0.845$, $P < 0.001$) and at 7mm points ($R \geq 0.654$, $P < 0.001$). A gradual increase in thickness was noted from the centre to the periphery with a high positive correlation between the CTL values and the mean thickness at the circles of 2,4,6,8 and 10 millimetres ($R \geq 0.635$, $P < 0.001$).

Conclusion:

The results suggest that central corneal thickness can serve as a good guide for predicting peripheral thickness. For surgical procedures specifically undertaken at mid-peripheral and peripheral zones, the actual measurements at the site of surgery may confer some advantage.

De-novo Malignancies after Tacrolimus Therapy in the Management of Ocular Inflammatory Diseases

Bhatt, U., Raj, D., Dua, H.S.

University Hospitals Nottingham

Purpose:

The appearance of "*de-novo*" tumors in adults receiving systemic immunosuppression is considerably greater than in the general population. The purpose of the present study was to analyze the incidence and type of malignancies in patients receiving long term Tacrolimus.

Methods:

Between Jan 1999 to Dec 2009, the case notes of all 212 patients who had received Tacrolimus as the immunosuppressive agent of choice in the management of a variety of ocular conditions such as high risk corneal grafts, uveitis, corneal stem cell allograft and scleritis were reviewed. Tacrolimus treatment dosage, schedule and duration of treatment were noted.

Results:

There were 16 patients (7.54%) who developed ≥1 malignancy after mean (±SD) duration of 45±26 months after the start of treatment. The skin malignancies included squamous cell carcinomas (n=2), basal cell carcinoma (n=2), and melanoma (n=1). The number of patients diagnosed with non-dermatological malignancies were as follows- prostate (n=3), lymphoproliferative (n=2), lung (n=2), bowel (n=1), kidney (n=1), ovarian (n=1), and breast (n=1). Multivariate analysis showed that the duration of treatment as the common statistically significant ($p<.005$) factor for both skin and non-skin malignancies.

Conclusions:

The incidence of *de-novo* malignancy in patients treated with Tacrolimus is many times higher than in the general population. It therefore demands careful long-term screening with high index of suspicion to facilitate the diagnosis and treatment in the early stages of a malignancy to improve patient survival.

Efficacy and Side-Effects of Hypertonic Sodium Chloride Ointment (5%) in the Treatment of Recurrent Corneal Erosions

Tsatsos, M., Savant, V., Prydal, J.

Leicester Royal Infirmary

Introduction:

Recurrent corneal erosions occur due to impaired regeneration or repair of the epithelial basement membrane and therefore delayed adhesion between the epithelium and anterior stroma. Most patients with recurrent erosions respond to topical lubrication therapy. It is our current practice to start treatment with hypertonic saline (NaCl 5%) ointment should initial treatment with topical lubrication fail.

To assess its effect and its potential side-effects on corneal endothelium we asked patients about the improvement of their symptoms and measured endothelial cell denisty before the initiation of treatment and 1 month after the start of hypertonic saline (5%) ointment twice a day.

Results:

All patients reported an improvement of their symptoms and specular microscopy showed no difference in the pre and post treatment endothelial cell counts.

Conclusions:

Hypertonic agents thought to promote the adherence of epithelial cells to the underlining tissues via the production of a transient osmotic gradient and therefore the absorption of fluid from the epithelium seem to offer a useful and safe alternative in the treatment of corneal erosions.

Research Presentation
Poster Abstracts

Poster Exhibition is located in the Conference Hall

No: 1

An Evaluation of the Usefulness of a Uniocular Trial of Topical Glaucoma Medication when Initiating Therapy

Uppal, S., Lakshmanan, A., Abedin, A., Henry, E., Rotchford, A.P, King, A.J.

University Hospital, QMC, Nottingham

Purpose:

To assess the value of a uniocular trial.

Methods:

Twenty eight phakic patients with untreated open angle glaucoma or ocular hypertension and IOP>21 mm Hg in both eyes were included. IOP was measured at 8am, 11am and 4pm on one day a week for three weeks. On the third week travoprost was started in the eye with the higher IOP, on week 4 treatment was started in the second eye and measurements were made over the following 3 weeks.

Results:

28 patients recruited

- 392 separate measurements at 11am

Regression to mean demonstrated in both eyes (V0 – mean V1,V2,V3)

Difference noted between adjusted and unadjusted IOP values for first eye

Significant reduction in IOP for both eyes with Travatan (true effect)

- First eye = 8.5mmHg [(meanV1,V2,V3) – (meanV5,V6,V7)]
- Second eye = 6.8mmHg [(meanV1,V2,V3) – (meanV5,V6,V7)]

No significant difference between adjusted IOP and true mean effect in first eye.

Discussion:

The value of a uniocular trial of topical glaucoma medication in predicting a response to medication is a controversial approach to commencement of therapy and the results in the literature are inconclusive. Our study shows that a uniocular trial of topical glaucoma medication results in a measurable reduction of pressure in the treated eye which remains when adjustment for regression to the mean is made.

Conclusion:

A uniocular trial is a useful clinical indicator of an individual's response to a glaucoma drop.

No: 2

A Comparative and Illustrative Discussion of Three-Dimensional Optical Coherence Tomography Imaging of Optic Nerve Pathologies

Gout, T., Gout, I. and Kheterpal, S.

Cambridge University Clinical School, Cambridge

Purpose:

Analyse and discuss the imaging of significant optic nerve pathologies using three-dimensional Optical Coherence Tomography (3D-OCT) in comparison with the present armamentarium of current imaging modalities.

Methods:

We assessed the following imaging modalities to illustrate, compare and contrast optic nerve pathology: 3D-OCT, 2D-OCT and colour imaging. Optic nerve pathologies of disc drusen, papilloedema and myopia are shown for comparison with the normal optic nerve head. A systemic literature review of OCT imaging of each pathological condition was undertaken searching the Medline database. Publication titles and abstracts were independently assessed for both relevance and quality in accordance with the CRD Guidelines.

Results:

The high-resolution of 3D-OCT images make them a powerful diagnostic tool, such as for the detection of early-stage NFL changes that may be visualised more clearly than with conventional imaging modalities. Current literature searches show growing interest in 3D-OCT application for optic nerve imaging, although this remains an ancillary imaging tool used in conjunction with conventional imaging modalities for a comprehensive pathological evaluation.

Conclusion:

3D-OCT of retinal pathology is widely documented in literature and applied in clinical practice. The full potential of 3D-OCT in the diagnosis, management and follow-up of optic nerve pathology remains to be realised.

No: 3

Diabetic Retinopathy Screening in Primary Care

Gkotsi, D. and Ratne, J.

Luton and Dunstable Hospital

Purpose:

Diabetic retinopathy is the leading cause of blindness in people under the age of 60 in industrialised countries, and a major cause of blindness in older people. Our GP Surgery in Luton serves a total practice population of 10,068 patients, of which 435 (4.32 %) are registered as diabetic. 134 patients have failed to attend retinopathy screening in the past 15 months. The Bedfordshire Diabetic Retinopathy screening is responsible for inviting practice patients for their diabetic retinopathy screening which is performed in Bedford Hospital. The aim was to identify the causes for the poor retinopathy screening attendance.

Methods:

The NICE guidelines were used as a gold standard. According to NICE all diabetic patients with no signs of diabetic retinopathy should have retinopathy screening annually and patients with worsening retinopathy or uncontrolled BM's, hypertension or renal impairment should have retinopathy screening every 3-6 months. We systematically reviewed the records of all diabetic patients who failed to attend retinopathy screening in the last 15 months and performed a telephonic survey in order to identify the reasons for their poor attendance.

Results:

Male patients were less compliant with the retinopathy screening than female patients, especially in the ages of 45-65. Among the non-compliant diabetic patients, a large portion had other risk factors for diabetic retinopathy: uncontrolled BM's (28.4%), hypertension (34.3%) and renal impairment (19.4%). The reasons for the poor attendance as identified by the telephonic survey were: mobility problems, inconvenience of the screening centre location (Bedford) which is a long distance from Luton, lack of correspondence from the screening centre due to incorrect patient contact details and finally low awareness of the importance of retinopathy screening.

Conclusions:

Lack of education, mobility problems and long distance from the screening centre are major factors for poor attendance at retinopathy screening. Consequently, the distribution of leaflets with information about diabetic retinopathy, home visits by eye specialists in order to cater for immobile patients and careful geographical selection of screening centres could improve retinopathy screening attendance.

No: 4

Safety of Reusable Prisms and Efficacy of Goldmann Tonometers

Abeywickrama, L., Dave, D., Nyarko, M., Mathew, M.

Department of Ophthalmology, Chesterfield Royal NHS Foundation Trust

Aim:

The safety of Goldmann applanation reusable prism was assessed by studying prisms which were less than two years, 2-10 years and those greater than 10 years old. The quality of IOP measurement was assessed by checking the calibration status of all tonometers in the department.

Methods:

All reusable prisms were assessed for manufacturer's lot numbers and the average age was identified. The condition of reusable prisms was graded by number of scratches, quadrants involvement, chips, and deposits. The calibration bar was used to assess the accuracy of tonometers with and without calibration seal by the MESU.

Results:

A total 8 re-usable prisms less than 2 years, 16 prisms between 2- 10 years and 16 greater than 10 years were studied. 6 calibrated tonometers were compared with 6 of those not calibrated. Prisms with an age of less than 2 years had significantly lesser number of scratches ($p< 0.001$) than those between 2-10 years of age. Re-usable prisms greater than 10 years had significantly higher number of scratches ($P<0.0001$), deposits and chips compared to those which were less than 10 years old. Further, calibrated tonometers had a lower error +/- 2 mm Hg than those without calibration (range 2-8 mm Hg)($p<0.001$).

Conclusions:

It is recommended for safety reasons; the Goldmann applanation reusable prism is replaced after an operational life of two years. The Goldmann applanation tonometer should be calibrated at regular intervals.

No: 5

Familial Presentation of Non-Specific Vertical Diplopia

Maconachie, G., Mclean, R., Sheth, V. and Gottlob, I.

University of Leicester

Purpose:

To describe a familial presentation of a vertical phoria with intermittent diplopia.

Methods:

We examined a family in which several members presented at clinic with a vertical phoria with non specific intermittent diplopia. A full orthoptics assessment on all family members and on the adult patients, eye movement recordings in different positions of gaze and a Harms wall was performed. Two symptomatic brothers had further tests including MRI scans, acetylcholine receptor antibody tests and thyroid profiles.

Results:

The examination of the family revealed no significant findings except for two brothers who both reported developing vertical diplopia starting within their twenties which occurs randomly and not every day. On examination a vertical phoria/tropia was found which was variable in size and reversed in direction in one of the brothers. Other clinical tests revealed no underlying cause of this deviation. The brothers also reported that the diplopia was aggravated by stress, tiredness and alcohol. The diplopia is currently controlled by Fresnel prisms of varying strengths which the brothers alternate between as needed. The mother reported similar symptoms when she was younger which sporadically improved when she became pregnant.

Conclusions:

We report a case study of a family with intermittent vertical phoria/tropia with intermittent diplopia presenting in their twenties. We did not find similar case reports in the literature.

No: 6

Alcohol Delamination of the Corneal Epithelium: Diagnostic and Therapeutic Applications

Said, D.G., Raj, A., Raj, D., Miri, A., Bhatt, U. and Dua, H.S.

University of Nottingham, Division of Ophthalmology and Visual Sciences

Aim:

Alcohol delamination (ALD) of the corneal epithelium is a useful technique for the management of recurrent corneal erosions. We explored the applicability of this technique to diagnose and/or treat clinical conditions where corneal epitheliopathy was the dominant feature.

Methods:

19 eyes of 17 cases were included. An optical zone marker was applied firmly to the cornea enclosing the area of pathology. The 'well' was filled with 20% ethanol for 30-40 seconds and the loosened epithelium was peeled off. The underlying stroma was washed with BSS and a bandage contact lens was applied. The epithelial sheet was spread out on a piece of non-absorbable paper, fixed with formalin or glutaraldehyde and processed for light or electron microscopy.

Results:

Patients' age ranged from 21 to 84 years. The clinical diagnosis was conjunctival intraepithelial neoplasia in (3); intraepithelial microcysts in (2, one bilateral); extensive mapdot fingerprint dystrophy (1, bilateral); metaplastic epithelium on cornea (3); superficial recurrence of granular dystrophy (3); Ries-Bucklers dystrophy (1) and indeterminate epitheliopathy (4). The diagnosis could be ascertained in 8 of 13 cases. In one case the sheet was fragmented and in one it was lost during processing. Improvement in vision was achieved in all patients with superficial dystrophies and resolution of indeterminate epitheliopathy was achieved in all cases.

Conclusions:

ALD allows biopsy of the corneal epithelium with retention of tissue orientation for accurate histological diagnosis as well a providing a cure for certain epitheliopathies without damaging Bowman's zone.

No: 7

Atypical Hydrops

Said, D.G, Miri, A., Maharajan, V.S. and Dua, H.S

University of Nottingham, Division of Ophthalmology and Visual Sciences

Aim:

To report 2 cases of previous decentred corneal grafts who had atypical oedema

Methods:

First case presented with unilateral hydrops affecting the host rim, but not involving the graft Second case presented with unilateral peripheral inferior oedema mainly in the host rim but also involving the lower part of the graft, she was put on prednisolone 0.5% 2 hourly for 3 weeks but the oedema continued to progress with drop of visual acuity to 6/60, Finally a Descemet stripping endothelial keratoplasty was done.

Results:

In the first case the Descemet's tear healed with resolving of the oedema over a period of 3 months with final best corrected visual acuity (BCVA) of 6/9, the second case, the oedema settled completely with an attached endothelial graft, her BCVA improved to 6/9.

Conclusion:

Atypical hydrops can occur in the host as well as from internal dehiscence at the graft host junction.

No: 8

Characterisation of Antimicrobial Peptides in Fresh versus Transplant-Ready Amniotic Membrane: Therapeutic Implications

Mathew, M., Hopkinson, A., Mohammed, I., Abedin, A., Suleman, H. and Dua, H.S.

University of Nottingham, Division of Ophthalmology and Visual Sciences

Aim:

Amniotic membrane (AM) has been used as a substitute for lost or damaged tissue on the ocular surface, providing an environment for improved healing to take place. Antimicrobial peptides (AMPs) are cationic host defence peptides with microbicidal properties providing a "chemical barrier". Antimicrobial activity of the amniotic membrane against bacterial pathogens was studied. We also determined the effect of preservation and processing on AMPs in fresh amniotic membrane (FAM), and transplant ready amniotic membrane (TRAM) samples.

Methods:

Antimicrobial assay was performed for FAM and TRAM by plate spotting method, minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) techniques against common bacterial ocular surface pathogens. Conventional PCR (RT- PCR) of 21 AMPs and subsequently Quantitative real-time polymerase chain reactions (QRT-PCR) for relative gene expression of AMPs detected by RT- PCR of 8 AMPs namely, HBD-1, HBD-2, HBD-3, LL37, Leap-1, Leap-2, DEFB109 and RNA-7 was performed. Further protein expression of these 8 AMPs in FAM and TRAM samples was studied by both immunohistochemistry and Western blot analysis.

Results:

Plate spotting revealed antimicrobial activity of AM against pathogens. MIC and MBC confirmed, FAM had greater activity against pathogens than TRAM samples*. QRT-PCR confirmed RT- PCR expression of the above 8 AMPs. Further, all 8 AMPs were detected by IHC in fresh samples. However, protein expression was decreased in TRAM samples in comparison to FAM, confirmed by Western blot.

Conclusions:

This is the first known study of the antimicrobial activity and profile of AMPs in fresh versus transplant ready amniotic membrane specimens. Processing appears to alter the AMPs protein expression in TRAM specimens.

*This data is preliminary and needs confirmation by further experimentation.

No: 9

An Excel Template for Monitoring of Patients on Immunosuppression Therapy

Nica, I., Tsatsos, M., Savant, V., Prydal, J.

Leicester Royal Infirmary, Leicester

The aim is to present specialised software for monitoring of immunosuppressive therapy, and describe its benefits and limitations.

While professional commercial programs are available for this purpose, they are expensive requiring yearly renewal of site licences. As necessary funding was not available, we developed a system based on Microsoft Excel spreadsheets, thus utilising software already available within the Trust and incurring no additional expense. Each patient is assigned an Excel file which is stored on a virtual shared drive on the hospital network. Access to the drive is limited to members of the corneal firms.

The spreadsheet shows at a glance the immunosuppressive drugs in use, any topical medication and a user definable list of results; this includes parameters from the full blood count, electrolytes, creatinine, liver function as well as inflammatory markers - as relevant to the disease in question and patient's treatment.

When an investigation result is outside the normal limits it conveniently appears in red, alarming the treating physician of the abnormal result. As well as patient data, the screen shows the main diagnosis as well as any additional relevant diseases or problems. Baseline creatinine is displayed along with the maximum permitted figure, which is relevant to patients on medication with potential renal toxicity. A second sheet is used to summarise investigations performed as a part of the diagnostic work up.

The software is not linked to the pathology database and thus results need to be entered by hand. However, it provides a clear display of variations in important indices, both in time and correlated with changes in medication.

No: 10

Profile of Sight Threatening Infectious Keratitis in Nottinghamshire; A Prospective Study

Otri, A.M., Fares, U., Al-Aqaba, M., Miri, A., Maharajan, S. and Dua, H.S.

Division of Ophthalmology and Visual Sciences, University of Nottingham

Aim:

To identify the predisposing factors, causative organisms, clinical presentation, treatment profile and potential complications of severe infectious keratitis at Queens Medical Centre in Nottinghamshire.

Methods:

Prospectively, all cases of serious infective keratitis which were presented to Queens Medical Hospital over a 3 year period were recruited. Detailed information about the clinical aspects, microbiological results and management profile were documented.

Results:

129 patients (143 eyes) equally distributed between females and males were prospectively enrolled in this study. The average age was 52.8 ±22.1 years. The most frequent risk factors were: ocular surface disease (32%), contact lens (CL) wear (26%) and previous ocular surgery (20%).

Staphylococcus aureus was the most common isolated bacteria (18.8%). Interestingly, *Acanthamoeba* was the second (16.6%) and *Pseudomonas aeruginosa* was the third (15%). Fortified cefuroxime/ gentamicin and PHMB/ Brolene were the mainstay of the treatment in bacterial and *Acanthamoeba* keratitis, respectively. Overall, 8.3% of cases needed corneal grafts. There was significant improvement in VA after treatment in the BK group.

Conclusion:

Previous ocular disease was the main predisposing factor for sight threatening infections keratitis. *Acanthamoeba* keratitis (AK) was a significant causative organism and showed almost ultimate correlation with soft CL wear. Old age, deep infiltration, steroid use, poor initial VA were risk factors for prolonged course of treatment in BK group, whereas deep infection was the only factor which increased the course of management in the AK group. Hot and cold corneal grafting are reasonable managements for severe corneal ulcers. Proper management of severe corneal infections is mandatory to prevent the devastating complications.

No: 11

Amniotic Membrane Use in Ocular Surface Burns

Allen, C.L., Clare, G., Hopkinson, A. and Dua, H.S.

Division of Ophthalmology and Visual Sciences, University of Nottingham

Introduction:

AM is a biomaterial extensively used in ocular surface reconstruction as a graft or a protective overlay to relieve pain and prevent chronic inflammatory sequelae, including neovascularisation and scarring. Current UK legislation in procurement and use of AM is lengthy and cryopreservation often renders the tissue non-viable and in an inadequate form for use in the battlefield. Freeze-dried (FD) AM however has been shown to retain its physical properties on reconstitution and has recently been used as an alternative in civilian ophthalmic surgery and by the US army. FD stabilises degradation and simplifies membrane storage, which has clear potential benefits for battlefield application.

Materials and Methods:

AM was collected from consenting patients undergoing elective caesareans. Sections of AM were subject to optimisation and FD prior to comparing the physical and biochemical properties to fresh and cryopreserved AM. Structural assessment was achieved using SEM and TEM. Samples were biochemically profiled using protein array technology and biomarker bioavailability was determined using immunofluorescence analysis.

Results and Conclusions:

Modifications to the FD process have been successfully optimised to produce AM for clinical application that is structurally and biochemically comparable to fresh than current cryopreserved material. Further experiments are being carried out to establish the beneficial potential of FD AM *in vitro,* using co-culture models mimicking acute injury and long term wound healing.

No: 12

A New "Hydrogel Bandage" Technique to Cover the Conjunctival-Limbal Wound in Fornix-based Trabeculectomy Surgery

Calladine, D., Ratnarajan, G., McAllister, J.

Prince Charles Eye Unit, King Edward VII Hospital, Windsor, UK

Purpose:

To investigate whether a hydrogel can be used to cover the conjunctival-limbal wound in fornix-based trabeculectomy surgery.

Methods:

A prospective consecutive case study was conducted of 8 patients undergoing trabeculectomy surgery. The conjunctival relieving incision was closed using 2 interrupted 10/0 nylon conjunctival/corneal sutures with buried knots, one placed at each end. A hydrogel was used to cover the conjunctival-limbal wound. Eyes were examined in the immediate postoperative period, at 1-day and 7-days following surgery. Coverage of the hydrogel was documented using colour slit lamp photography, intraocular pressure measured using applanation tonometry and a Seidel test performed.

Results:

At the end of surgery and in the immediate postoperative period the hydrogel fully covered the conjunctival-limbal wound in all cases. At the day-1 examination the hydrogel had full coverage of the wound in 2 out of 8 cases and partial coverage in 6 cases where it selectively adhered to areas of tissue damage. At the day-7 examination 2 cases had partial coverage localised to areas around the 2 sutures and in the remaining 6 cases the hydrogel had gone. All wounds were Seidel negative with no cases of hypotony.

Conclusions:

This study demonstrates how a "hydrogel bandage" technique can be used to cover the limbal-conjunctival wound in fornix-based trabeculectomy surgery. The authors believe this technique helps the wound seal and is particularly useful for elderly patients with thin fragile conjunctiva and in cases when anti-metabolites are used.

No: 13

Analysis of Iris Abnormalities in Albinism using Anterior Segment Optical Coherence Tomography

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Purpose:

Albinism is associated with visible abnormalities of the iris such as transillumination. Recent developments in anterior segment optical coherence tomography (AS-OCT) now permit accurate imaging of iris anatomy. We have used AS-OCT to compare iris thicknesses of patients with albinism to healthy control subjects. We have also explored the relationship between iris thickness and iris transillumination in the albinism group.

Methods:

We imaged the irides of 51 patients with albinism, diagnosis confirmed by abnormal VEP crossing, using high resolution AS-OCT. These were compared to 31 healthy control subjects. All individuals were illuminated using a bright white light source to constrict the pupils. Iris thicknesses were measured along cross sectional iris images using ImageJ software and thicknesses were averaged at 111µm intervals. Iris thicknesses were compared to clinical iris transillumination severity using the Summers classification scheme. Comparisons between groups were made using ANOVA.

Results:

Mean iris thicknesses were significantly thinner in albinism patients compared to the healthy control group ($p<0.001$). This difference was apparent along the whole iris profile. In thirty out of the fifty-one albinism patients iris thickness fell below the 95% confidence interval of control subjects There was no significant correlation between clinical iris transillumination severity and the iris thickness measured with AS-OCT in the albinism cohort ($p<0.05$).

Conclusions:

Through the use of the AS-OCT we have shown that iris thickness is significantly reduced in albinism patients compared to the healthy controls. AS-OCT may assist in the diagnosis of albinism with 59% of iris thicknesses in albinism patients falling outside that of normal values.

No: 14

Validation of Endogenous Control Genes for Normalisation of Real Time PCR on OS Epithelial Regions

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Introduction:

The real time PCR is the most popular validation tool in genomic studies. With this method the quantitative expression of gene of interest is normalised relative to the endogenous control gene. The aim of this study is to identify stably expressed endogenous control genes for normalisation of genes of interest determined from ocular surface (OS) gene ST 1.0 array study.

Methods

The OS epithelial regions studied were cornea, limbus, LEC, conjunctiva. The amplified cDNA from Gene ST 1.0 array study was also used to generate samples for validation of endogenous control genes and also for real time PCR of genes of interest. Expression of ten known endogenous control genes such as HPRT, PPIA, TBP, GUSB, 18S, GAPDH, B2M, ACTB, PGK1, RPLPO were determined on OS epithelial regions and further analysed with GeNorm software.

Results

The stably expressed genes were determined by calculation of geometric mean with geNorm software. Normalisation of gene of interest with individual endogenous control genes and with normalisation factor derived from the stable pair of endogenous control genes was compared. The gene expression levels were significantly affected by the selected endogenous control gene.

Conclusion

This study has identified stably expressed pairs of endogenous control genes on the OS epithelial regions for reliable normalisation of genes of interest.

No: 15

The Morphology of Corneal Nerves in Advanced Keratoconus

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Purpose:

To study the morphology of corneal nerves in patients with advanced keratoconus.

Methods:

Fourteen corneal buttons from 14 keratoconic patients (9 males, 5 females; mean age, 34.3) who had keratoplasty for advanced keratoconus and 6 normal control corneal buttons were recruited. All specimens were stained as whole mounts with acetylcholinesterase (AchE) method for corneal nerve demonstration and scanned in multiple layers with NanoZoomer Digital Pathology scanning microscope.

Results:

Seventy one percent of corneas in the study group demonstrated central stromal nerve changes including thickening, tortuosity and abnormal nerve sproutings. The thickness of central stromal nerve was 18.9±14.7 μm which was thicker than that in the controls (8.11±3.31 μm) (*p=0.000*). The thickness of peripheral stromal nerves was 12.6±3.1 μm and was not statistically different from the peripheral stromal nerve diameter in the controls (14.86±5.60 μm) (*p=0.072*). All corneas demonstrated loss of normal sub-basal nerve architecture. Sub-basal nerves showed loss of their radial orientation and increased tortuosity mainly at the centre of the cone. At the border of the cone, these nerves tended to run in concentric rings. Localized thickenings of sub-basal nerves were often observed at their origin from the bulb like structures just above the Bowman's zone.

Conclusions:

Significant alterations in corneal innervation are present in corneas with advanced keratoconus. This study provides histological confirmation of the involvement of corneal nerves in the pathology of keratoconus.

No: 16

Correlation of Central and Peripheral Corneal Thickness in Healthy Corneas

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Introduction/Purpose:

To study the thickness profile of the normal cornea in order to establish any correlation between central and peripheral points.

Methods:

Sixty seven eyes of 40 patients were subjected to central corneal thickness measurement (CCT) with an ultrasound pachymeter (UP) and corneal thickness mapping with the Oculus Pentacam. The corneal apex thickness (CAT), pupil centre thickness (recorded as CCT and corresponded to CCT of UP) and thickness at the thinnest location (CTL) were obtained and compared with each other. Corneal thickness data at 3mm and 7mm temporally, nasally, superiorly and inferiorly from the corneal apex were obtained. The mean corneal thickness values along the 2,4,6,8 and 10 millimetres diameter concentric circles, with the CTL as the centre, were also obtained. The above data at different points were statistically correlated by Pearson product-moment correlation coefficient.

Results:

There was no significant difference between CCT readings measured by UP and Pentacam *(p=0.721)*. There was high positive correlation between the CAT values and the thickness at 3mm ($R \geq 0.845$, $P<0.001$) and at 7mm points ($R \geq 0.654$, $P<0.001$). A gradual increase in thickness was noted from the centre to the periphery with a high positive correlation between the CTL values and the mean thickness at the circles of 2,4,6,8 and 10 millimetres ($R \geq 0.635$, $P<0.001$).

Conclusion:

The results suggest that central corneal thickness can serve as a good guide for predicting peripheral thickness. For surgical procedures specifically undertaken at mid-peripheral and peripheral zones, the actual measurements at the site of surgery may confer some advantage.

No: 17

Limbal Stem Cell Isolation and Characterisation for *ex vivo* Transplantation

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Introduction:

The corneal epithelium is a self-renewing tissue that maintains its integrity, important for vision by harbouring stem cells at limbal region. Their depletion leads to conditions such as limbal stem cell deficiency (LSCD). A treatment options for LSCD involves transplantation of limbal stem cell (LSC) *ex-vivo* expanded on a carrier substrate. In vitro studies of 'limbal stem cells' often use primary cells expanded from a limbal explant or a single cell suspension, or corneal epithelial cell lines, with the results often comparable without concern. However, differences between the cell types due to varying culture and environmental condition are not fully considered. Therefore, we undertook molecular study to compare the cell 'state' of primary limbal cultures and corneal epithelial cell line with the aim of characterising key similarities and differences.

Methods:

The corneoscleral rims were used for limbal explant culture. RNA isolated from limbal explant and corneal epithelial cell line was reverse transcribed to cDNA Quantitative real time PCR using Taqman probe was used to study the gene expression for limbal stem cell (LSC) markers CK19,ABCG2,P63,SOD2, HES1,FRZB1 and terminally differentiated CK3,CK12,DSG3.

Results:

Gene expression profile from explants culture showed more differentiated phenotype by high expression level for CK3, CK12 and DSG3 whilst retaining expression of stem cell p63, ABCG2, FRZB1, HES1. The corneal epithelial cell line showed undifferentiated phenotype by lack of CK3, CK12, DSG3 and presence of CK19, SOD2, HES1, FRZB1,ABCG2 gene expression but no expression of p63.

Conclusion:

Explant culture appears to be mostly differentiated but still retaining stem cell like profile. This shows the heterogeneous population from limbal explants. This may explain poor longevity following clinical transplantation of *ex-vivo* expanded limbal explants. Cell line and primary explant culture are very different and care should be taken to validate cell profile before using in research.

No: 18

Transdifferentiation of Human Mesenchymal Stem Cells into Corneal Epithelium

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Introduction

Mesenchymal stem cells (MSC) can differentiate into a variety of cell types, and MSC-epithelial transdifferentiation has been shown *in vitro*. Regeneration of the ocular surface using transplanted MSC has been successful in animal models. In humans this treatment may mitigate many of the disadvantages associated with limbal stem cell transplantation for severe ocular surface disease. However it is crucial to first establish the potential of MSC to differentiate into corneal epithelial cells (CEC).

Methods

A heterogeneous population of MSC was extracted from human foetal liver. Single random isolates from the heterogeneous MSC population were clonally expanded. These clonal MSC were then stimulated in specific medium to transdifferentiate into CEC. Cell morphology was analyzed by light microscopy. Limbal stem cell (LSC) markers (cytokeratins14 & 19, ABCG2, vimentin and P63) and terminally differentiated CEC (cytokeratin3/12, E-cadherin, connexin43) were analyzed by flow cytometry and qPCR.

Results

Some, MSC clones exhibited complete transdifferentiation whereas others retained their MSC phenotype. The transdifferentiated MSC expressed both LSC and CEC markers.

Conclusion

Human MSC can transdifferentiate into LSC and CEC *in vitro.* LSC and CEC appear to only be generated by specific MSC sub-populations. Pre-selection of MSC sub-populations before transplantation onto the ocular surface may expedite corneal regeneration.

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