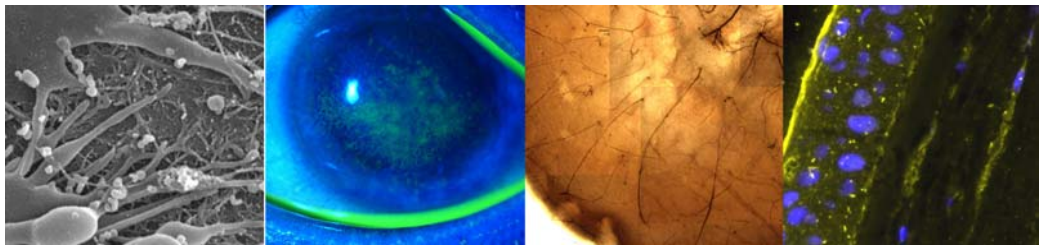
The 14th Nottingham Eye Symposium
and Research Meeting

featuring
The Norman Galloway Lecture



Programme and Abstracts

Friday 29th January 2010

The University of Nottingham

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

The 15th Nottingham Eye Symposium and Research Meeting
***featuring the Norman Galloway Lecture* will be held on**
Friday, 28th January 2011 (provisional date)

- Research trainee abstract presentations (oral and poster) and prizes
- Guest presentations by leading optometrists
- A symposium with talks from prestigious ophthalmologists
- The Norman Galloway Lecture
- Excellent conference facilities including free parking
- Tea, coffee and a hot buffet lunch provided
- All for a very reasonable registration fee!

Details will be circulated in September 2010.

Contact the NES Meeting Co-ordinator: nes@nottingham.ac.uk or check out the website: http://www.nottingham.ac.uk/scs/divisions/ophthalmologyvisualsciences/nes/nottinghameyesymposium for details of next years meeting.

Contents

No: 19

Denuded Amniotic Membrane in Corneal Epithelial Cell Expansion

Yeung, A.M., Mohammed, I., Tint, N.L., Hopkinson, A. and Dua, H.S.

University of Nottingham

Introduction
The Amniotic Membrane (AM) has grown in popularity over the years as a substrate for epithelial expansion and treatment of LSCD. The AM basement membrane (BM) provides the necessary structure for cell attachment, and the AM itself is postulated to possess anti-inflammatory and anti- scarring properties. In this study we compared the potential for expansion of primary epithelial cells from explants between intact and denuded AM.

Methods
Corneal-scleral explants were cultured on both intact and denuded Amniotic Membrane. Primary cornea epithelial cells were cultured for 12 days and tissue were collected for 1) immunohistological analysis and 2) real time q-PCR.

Results
Denuded AM permitted greater stratification of epithelial cells with a higher expression of Tenascin C staining. In addition, these cells demonstrated a greater expression of p63 and ABCG2 and lower expression of Desmoglein 3 and was confirmed at a genomic level by qPCR.

Conclusion
Denuded AM permitted expansion of primary epithelial cells that demonstrated characteristics with a more "stem like" nature.

No: 18

The i*n vitro* Effects of Anti-Angiogenic Treatments on Human Choroidal Endothelial Cells

Stewart, E.A., Amoaku, W.M.

Division of Ophthalmology and Visual Sciences, University of Nottingham

Purpose: Age-related macular degeneration (AMD) is the most common cause of irreversible visual loss in elderly populations in the western world. There are 2 types: wet and dry AMD. Wet AMD is secondary to neovascularisation from the inner choroid, called choroidal neovascularisation (CNV). However, the mechanisms responsible are not fully understood. Although several growth factors have been identified in CNV, vascular endothelial growth factor (VEGF) is thought to be the most significant growth factor in wet AMD. As such, molecular inhibitors of VEGF, including pegaptanib (Macugen), ranibizumab (Lucentis) and bevacizumab (Avastin) have been developed as treatments for wet AMD. Direct clinical comparison of the efficiency of the drugs is not available, due to variations between trials and dosing regimes. The purpose of this study was to evaluate the *in vitro* anti-angiogenic effects of the 3 agents currently available.

Methods: hCEC were isolated from cadaver eyes and cultured in EBM2-MV medium as previously published by this laboratory. Proliferation of un-passaged, primary hCEC after exposure to VEGF isoforms 121 and 165 and anti-angiogenic treatments (pegaptanib (Macugen), ranibizumab (Lucentis) and bevacizumab (Avastin)) was measured using the WST-1 cell proliferation assay.

Results: VEGF isoforms 121 and 165 were found to be equally potent in the stimulation of hCEC proliferation and proliferation of other ocular cells. Both Ranibizumab (Lucentis) and bevacizumab (Avastin) were effective in decreasing proliferation of hCEC stimulated with VEGF isoform 121 or 165. Pegaptanib (Macugen) was moderately effective in controlling the proliferation of hCEC stimulated by VEGF 165, but was ineffective against the stimulatory effect of VEGF 121. When compared directly the efficacy of these drugs in vitro against a combination of VEGF 121 and 165 was in the order Lucentis>Avastin>Macugen.

Conclusions: Although molecular inhibitors of angiogenesis have been shown to have a beneficial effect as treatments for AMD, direct comparison of the efficiency of the drugs is complicated, due to variations between trials and dosing regimes. *In vitro* testing of the drugs on the proliferation and angiogenesis of hCEC showed that the efficacy is in the order Lucentis>Avastin>Macugen, in line with the most clinical outcomes seen.

Programme Summary

08.30 - 09.00:	Registration
09.00 - 09.05:	Chairman's Welcome and opening remarks
09.05 – 10.25:	Research Presentations *Chairman Prof HS Dua*
10.30 – 10.50:	Living with acquired visual impairment: Dr K Latham, Anglia Ruskin University
10.50 – 11.15:	Coffee Break (Poster and Trade Exhibitions)
11.15 – 12.10:	Research Presentations *Chairman Mr WM Amoaku*
12.10 – 12.30:	Are amblyopia & strabismus disabling conditions? DR B T Barrett, Bradford University
12.30 – 13.30:	Lunch Break (Poster and Trade Exhibitions)
13.30 – 14.15:	The Norman Galloway Lecture. Professor David Wong, University of Liverpool and Hong Kong

Symposium on Ocular Trauma
Chairman Prof HS Dua

14.15 – 14.35:	Epidemiology of ocular trauma: Dr CJ MacEwen, Dundee
14.40 – 15.00:	Corneal trauma and repair: Prof. HS Dua, Nottingham
15.05 – 15.25:	Oculoplastic trauma & reconstruction: Mr R Sampath, Leicester
15.30 – 15.55:	Tea Break (Poster and Trade Exhibitions)
15.55 – 16.15:	Posterior segment trauma: Professor D Wong Universities of Liverpool and Hong Kong
16.20 – 16.40:	War Trauma: Wg Cdr RAH Scott, RAF, Selly Oak, Birmingham
16.45 - 16.50:	Research Presentation and Poster Prizes
16.50 -16.55:	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

No: 17

Psychological Distress in People Disfigured by Facial Palsy

Fu, L.[1], Bundy, C.[1], Sadiq, S.A.[2]

University of Manchester[1], Manchester Royal Eye Hospital[2]

Purpose
Psychological distress is well documented in people living with facial disfigurement. However the prevalence of psychological distress in facial palsy patients has not been studied.
This preliminary study aims to establish the beliefs about their condition and level of psychological distress (extent of anxiety and depression) in a sample of patients with facial palsy from the Northwest of England.

Method
39 participants with facial palsy completed a questionnaire pack comprising of the Illness Perception Questionnaire-Revised (IPQ-R), demographic questionnaire, and Hospital Anxiety and Depression Scale. The severity of participants' facial palsy was measured by the House-Brackmann scale. Analysis was undertaken with SPSS 15 comprising descriptive, correlations, and comparisons of groups.

Results
Of the study sample, 17.9% and 23.1% had significant levels of anxiety and depression respectively. The rest had lower levels of distress that did not reach caseness according to the HAD scale. The prevalence of anxiety and depression in the normal population are 10.6% and 12% respectively. The mean age of participants was 61, with 52.8% having grade 6 facial palsy. Significant associations were found between participants' perception of consequences and emotional representations and the level of distress. There were no significant associations between clinical severity and levels of distress.

Conclusion
There was a significant level of distress in this study group. The levels of psychological distress were higher than the levels found in the normal population. There were significant associations between participants' illness perceptions and their level of distress but distress was not related to grade of palsy.

No: 16

Masked Hydroxychloroquine Related Toxic Maculopathy in Franceschette Naegeli Jadassohn syndrome: an Unusual Presentation

Kulkarni, A.R., Joseph, A.

The University Hospital, North Staffordshire

Introduction
Naegeli –Franceschetti- Jadassohn syndrome (NFJS) is rare and known to cause cutaneous hyperpigmentation and deafness. Maculopathy associated with NFJS has not been reported before. The pigment abnormality with FNJ syndrome may be a predisposition to macular toxicity with normal doses of hydroxychloroquine and related drugs.

Method:
We describe an unusual case of a 27-year old lady with Naegeli –Franceschetti-Jadassohn syndrome (NFJS) who developed gross visual loss secondary to toxic maculopathy with ≤6.5mg/kg/day of hydroxychloroquine for 8 years. The dose was within the recommended range. Her visual loss was attributed to her rare inherited pigmentary cutaneous disorder and resulted in the delayed recognition of hydroxychloroquine related toxic maculopathy. Visual fields, fundus fluorescein angiography and electrodiagnostic tests were performed.

Results:
The occurrence of toxic retinopathy to recommended doses of hydroxychloroquine emphasizes the unpredictable toxic effects of the drug in rare reticular epithelial syndromes possibly causing an increased affinity of the drug to bind to melanin.

Discussion:
Although screening for hydroxylchloroquine toxicity has not been found cost effective each case should be considered individually in order to limit the risk of severe visual loss. As seen in our case with NFJS there is a continued need for diligence amongst rheumatologists to ensure suitable testing at appropriate intervals for ocular toxicity. This has not been reported before. An increased risk of hydroxychloroquine related maculopathy should be considered in syndromes with pigment disorders.

Previous Norman Galloway Lectures

2009 **The Good, the Bad and the Ugly: The Metastatic Potential of Uveal Melanoma** Prof I G 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 J K G Dart, Consultant Ophthalmologist, 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, Professor of Ophthalmology, 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 C N J McGhee, Professor of Ophthalmology, University of Auckland, New Zealand

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

2001 **Classification and Treatment of Posterior Uveitis** Professor J V Forrester, Professor of Ophthalmology, University of Aberdeen

2000 **Herpes Simplex Viral Keratitis : What HEDS (Herpetic Eye Disease Studies) has taught us** Professor P R Laibson, Director – Cornea Service, Wills Eye Hospital, Philadelphia, United States of America

1999 **Management of Traumatic Ptosis** Mr J R O Collin, Consultant Ophthalmic Surgeon, Moorfields Eye Hospital, London

1998 **Stargardt's Macular Degeneration** Professor L A Donoso, Director of Research/Thomas D Duane Professor of Ophthalmology, Wills Eye Hospital, Philadelphia

1997 **Diabetic Retinopathy – a tolerable disease** Professor D B Archer Professor of Ophthalmology, 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)

Programme

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

9.05 am **Research presentations:**
Chairman: Prof. HS Dua, Nottingham

9.05 am Corneal Biomechanical Parameters in Asymmetrical Glaucoma
Poostchi, A., Henry, E. and Vernon, S.A. (University Hospital Nottingham)

9.12 am Comparative Transcriptional Profiling of Limbal Epithelial Crypt with other Ocular Surface Epithelium with Gene ST 1.0 Array
Kulkarni, B., Arno, M., Aldecoa-Otalora Astarloa, E., Hopkinson, A. and Dua, H.S. (University of Nottingham; King's College, London)

9.19 am Biomechanical properties of amniotic membrane preparations for ophthalmic surgery
Clare, G., Allen, C., Hopkinson, A. and Dua, H.S. (University of Nottingham)

9.26 am Differentiation status of limbal epithelial cells cultured on intact and denuded amniotic membrane before and after air-lifting
Chen, B., Mi, S., Wright, B. and Connon, C.J. (University of Reading)

9.33 am Amniotic membrane transplantation on the ocular surface: A Meta-Analysis
Suleman, H., Lazutina, E., Gicquel, J.J., Yeung, A.M., Mohammed, I., Tint, N.L., Desai, P. and Dua, H.S. (University of Nottingham; University of Poitiers, France; Moorfields Eye Hospital, London)

9.40 am Expansion of Human Mesenchymal Stem Cells on Amniotic Membrane for Use in Occular Surface Repair
Branch, M., Hopkinson, A., Jones, R. and Dua, H.S. (University of Nottingham)

9.47 am Inadvertent total loss of Descemet's membrane in a Deep anterior lamellar keratoplasty-an unusual complication
Ramamurthi, S., Obi, E., Mantry, S., Ramaesh, K. (Gartnavel General Hospital, Glasgow)

9.54 am Thermoreversible PLGA-PEG-PLGA Copolymer Hydrogel for Ocular Surface Reconstruction
Tint, N.L., Dhillon, A., Shakesheff, K.M., Dua, H.S. and Rose, F.R. (University of Nottingham)

10.01 am Tissue engineering of the ocular surface using plastically compressed collagen gels
Mi, S., Chen, B., Wright, B., Connon, C.J. (University of Reading)

10.08 am Architecture and Distribution of Human Corneal Nerves
Al-Aqaba, M.A., Fares, U., Suleman, H., Lowe, J. and Dua, H.S. (University of Nottingham)

10.30 am **Guest Lecture:**
Living with acquired visual impairment
Dr K Latham, Anglia Ruskin University

10.50 am to 11.15 am ***Refreshments; Poster Exhibition and Trade Exhibition***

No: 15

Wet-preserved versus Freeze-dried Amniotic Membrane in Ocular Surface Reconstruction

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

Division of Ophthalmology & Visual Sciences, University of Nottingham

Introduction: Human amniotic membrane (AM) is extensively used in ocular surface reconstruction. The properties of AM promote wound healing, prevent scarring, it acts as a substrate for cell growth and migration, inhibits vascularisation and has antimicrobial effects. Current UK legislation in procurement and use of AM is lengthy and wet preservation often renders the tissue non-viable following thawing, with the depletion of beneficial soluble factors. Freeze-dried (FD) AM however has been shown to retain its physical properties on reconstitution. Sequentially this would simplify storage and stabilise membrane degradation. This study was designed to optimise and standardise the FD process and characterise its effects on AM structure and biochemical composition versus wet-preserved.

Materials and Methods: AM was collected from consenting patients undergoing elective caesareans. AM (5cm^2) sections were treated with glycerol/PBC (20%), and trehalose (10%) in the presence or absence of epigallocatechin (1mg/mL) prior to FD. Some of these samples were structurally assessed using SEM and TEM. Soluble EGF protein concentrations were additionally assessed in samples and washes following processing and reconstitution, to ascertain the ability of the treatments in preventing factor depletion. In parallel a panel of biochemical factors were assessed in protein extracted from fresh, wet-preserved, denuded and FD AM using SearchLight immunoassay technology.

Results and Conclusions: Modifications to the FD process have been successfully optimised to produce a uniform layer of AM for clinical application. Further analyses of experiments performed are required to establish the effect of FD on factor retention versus wet-preserved AM.

No: 14

Peripheral ulcerative keratitis (PUK) in X-linked ichthyosis

Agarwal, P., Livingstone, I., Mantry, S

Glasgow

Purpose: Report the unusual presentation of a peripheral ulcerative keratitis (PUK) in a patient with X-linked ichthyosis.

Methods: PCR and Southern blotting techniques confirmed the diagnosis of X-linked ichthyosis. Full blood screen investigating other possible aetiologies of PUK (full blood count, urea and electrolytes/biochemistry, ENA, anti-Ro, anti-La, ds-DNA, serum ACE, ANCA, ANA, RhF) yielded negative results.

Results: A 62 year old male with a clinical diagnosis of ichthyosis presented to our clinic with a history of painless decline in vision in the left eye. Ophthalmic exam evidenced marked bilateral 360 degree corneal thinning, with findings consistent with peripheral ulcerative keratitis. The more typical findings of posterior stromal, flour-like granular opacities were not evidenced. The diagnosis of X-linked ichthyosis was confirmed via genetic analysis. He was managed with a tapering dose of oral steroid, ocular lubricants, and a 6-week course of oral tetracycline. His corneal appearances remain stable four months on from presentation.

Conclusions: Various corneal abnormalities have been reported in the context of ichthyosis (including exposure-related keratitis, unilateral megalocornea, recurrent erosions, Salzmann nodules, enlarged corneal nerves, stromal vasculaisation, limbal stem-cell deficiency), with posterior stromal opacities overwhelmingly the most common. The mechanism of corneal involvement is a subject of continued research. The finding of peripheral ulcerative keratitis has not, to our knowledge, been reported in the literature in the context of confirmed X-linked ichthyosis.

11.15 am **Research Presentations *(continued):***
Chairman Mr WM Amoaku

11.15 am Study of analgesic effectiveness of topical drop anaesthesia for intravitreal injection of ranibizumab
Puri, P., Trivedi, D., Moodie, J. (Derby Royal Hospital)

11.22 am Infectious keratitis profile in Nottinghamshire
Otri, A.M., Maharajan, S., Dua, H.S. (University Hospital, Nottingham)

11.29 am A study to establish the ocular and systemic safety of simultaneous bilateral intravitreal ranibizumab injections for wet AMD
Trivedi, D., Moodie, J., Puri, P. (Derby Royal Hospital)

11.36 am Childhood unilateral leucocoria in amateur photography
Fincham, G.S., Keightley, S.J., Sandy, C.J., Morsman, C.D. (Addenbrooke's Hospital, Cambridge)

11.43 am High resolution spatial and temporal expression profile of FRMD7 in neuronal tissue provides clues for pathogenesis and treatment
Thomas, M.G., Araki, M., Gottlob, I. (University of Leicester; Nara University, Japan)

11.50 am Vitreous length as a predictor of refractive outcome after penetrating keratoplasty for keratoconus
Fares, U., Al-Aqaba, M.A., Miri, A., Otri, A.M. and Dua, H.S. (Queen's Medical Centre, Nottingham)

11.57 am Adherence to RCOphth guidelines in monitoring patients on hydroxychloroquine by Rheumatologists
Sandhu, J., Joseph, A., Packham, J. (University Hospital of North Staffordshire)

12.04 pm Phacoemulsification and toric intraocular lens insertion for correction of post penetrating kearatoplasty astigmatism
Murjaneh, S., Smith, G.T. (The Great Western Hospital, Swindon)

12.10 pm **Guest Lecture:**
Are amblyopia & strabismus disabling conditions?
Dr BT Barrett, Bradford 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 14th Norman Galloway Lecture "East and West"**
Professor David Wong, University of Liverpool and Hong Kong

Symposium on Ocular Trauma *Chairman Prof HS Dua*

2.15 pm **Epidemiology of ocular trauma**
Dr CJ MacEwen, Dundee

2.40 pm **Corneal trauma and repair**
Prof. HS Dua, Nottingham

3.05 pm **Oculoplastic trauma & reconstruction**
Mr R Sampath, Leicester

3.30 pm to 3.50 pm ***Refreshments; Poster Exhibition and Trade Exhibition***

3.55 pm **Posterior segment injury**
Professor D Wong Universities of Liverpool and Hong Kong

4.20 pm **War Trauma**
Wg Cdr RAH Scott, RAF, Selly Oak, Birmingham

4.45 pm **Research Presentation and Poster Prizes**

4.50 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

Please turn in your name badge for recycling

See you next year!

No: 13

Proteomic Characterisation of Amniotic membrane(AM) Denuded using Different Techniques for Tissue Engineering

A. Hopkinson[1], S Liddell[2], C. Allen[1], K. Hashmani[1], H.S. Dua[1].

[1]Division of Ophthalmology & Visual Sciences, [2]Animal Sciences, University of Nottingham

Purpose:
Transplantation of Amniotic Membrane (AM) engineered with *ex vivo* expanded corneal limbal epithelium is an accepted modality for ocular surface reconstruction. However the techniques used for preparing AM have yet to be fully standardised. Several methods for denuding AM have been reported, of which the novel technique employing thermolysin appears to be the most standardised and reproducible for preserving an intact basement membrane, which is essential for maintaining stem/progenitor cells and promote cell growth. However, the full effects of denuding using different techniques on AM biochemical and structural composition and consequently the key proteins involved in cell expansion are unknown. We therefore employed proteomic analysis to investigate this further.

Methods:
Transplant Ready AM (TRAM) was denuded using published EDTA (AM^E), dispase (AM^D) and thermolysin (AM^T)-based methodologies. Differential 2-Dimensional electrophoresis was performed on proteins extracted from the 4 types of AM. Proteins of interest were immuno-detected using western blots and histological staining. Expansion of CEC on the different membranes was performed and measured to assess cell growth followed by histological detection of proteins of interest to evaluate potential cell-matrix interaction.

Results:
AM^E disintegrates and disperses but does not fully remove epithelium. AM^D removes epithelium but results in extensive digestion of AM structural proteins. Denuding using AM^T resulted in reproducible and limited digestion of selected structural proteins, such as collagen VI and VIII, BIGH3, and integrin alpha 6. Despite this, a significant proportion of the parent protein remained. Analysis of AM^T revealed the major structural components of AM extracellular matrix which include Lumican, Mimcan, Decorin, BIGH3, OSF2 which all have a role in cellular adhesion and growth, and the structural protein Collagens VI. Analysis of cell growth revealed that growth was most consistent on thermolysin treated membranes.

Conclusion:
AM^T is the most effective reported denuding technique. AM^E and AM^D are ineffective methods for denuding. Thermolysin exhibits specific activity against hemidesmosomal proteins (Integrin alpha 6), which may explain its mechanism for effective cellular release, with minimal damage to the BM or extracellular matrix. The enzymatic activity of thermolysin is limited such that it does not effect the localisation or bioavailability of potential proteins of interest. Therefore, AM^T is ideal for *ex-vivo* engineering of AM/epithelial constructs for improved clinical care.

No: 12

Antimicrobial properties of Amniotic Membrane and Spongy Layer

Lazutina, E., Suleman, H., Hopkinson, A. and Dua, H.S.

Division of Ophthalmology and Visual Sciences, University of Nottingham

Introduction
Spongy Layer (SL) is the gelatinous, proteoglycan rich layer between avascular amnion and vascular chorion collectively known as the foetal membrane. SL forms between day 6 and week 12, during the period of gestation in chorionic cavity when the amniotic membrane fuses with the chorion. Little is known about the function of SL, in-vivo. Even less known about the structure and biochemical composition of the SL. The current knowledgebase of the SL has been established indirectly through work carried out to assess the structural composition of the foetal membrane. SL is a proteoglycan rich structure, which can imbibe water and swell, a property thought to be a function to facilitate or "lubricate" mutual sliding of AM and chorion part of the short-term mechanical repair system. The SL also contains high levels of hyaluronan, a major carbohydrate component of the extracellular matrix (ECM) that is known to provide mechanical support and to interact with different growth factors. Antimicrobial properties of Amniotic Membrane (AM) are well known, however, none mentions antimicrobial properties of Spongy Layer (SL) as an independent layer.

Methods
To investigate the antimicrobial properties of SL minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) assays were used. *S. aureus, S. epidermicus*, Diphtheoid and Maxarela MIC and MBC were determined with different concentrations of SL extract.

Results
In these studies SL was found to be strongly bacteriostatic and therefore have antimicrobial properties as an independent layer.

Discussion
As previously reported, SL has strong eukaryotic cytotoxic effects and has now been shown to be bacteriostatic; however, SL is a depot of varying factors with extensive potential effects on cells. It is likely that SL is responsible for the extensive but variable biochemical composition of transplanted AM and maybe key for the reported variable clinical effects of the membrane.

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.

2009 Voluntary modulation of involuntary eye movements during reading
M G Thomas, F A Proudlock, R J McLean, I Gottlob
2008 Glaucoma in an elderly Caucasian population (The Bridlington Eye Assessment Project)
A Bhan-Bhargava; S A Vernon; R Jayaswal; V Owen; M Boraik, J A Hillman; H MacNab; J Van Der Hoek; P Bacon, P Redmond
2007 Digital infrared pupillometry for comparing cocaine with apraclonidine testing when investigating Horner's syndrome
A Shwe-Tin; G T Smith
2006 Linear regression modeling of rim area to discriminate between normal and glaucomatous optic nerve heads: The Bridlington eye assessment project.
M J Hawker; S A Vernon; C L Tattersall
2005 Can patching be improved in amblyopia treatment?
M Awan; I Gottlob; M Dixon-Woods
2004 Amniotic membrane transplantation for ocular surface reconstruction: A seven year retrospective analysis.
V S Maharajan; R S McIntosh; J McElveen; A Browning; H S Dua
2003 Effect and compliance of strabismic amblyopia monitored with the occlusion dose monitor
M Awan; F Proudlock; I Gottlob
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; R Bhola; J Bush; S Winder; J F Talbot
2001 The detection of T-Cell activation by retinal autoantigen in uveitis patients using cytokine flow cytometry
J Morgan; R A Robins; H S Dua; P H Tighe
2000 Spatial localisation in esotropia - is extraocular muscle proprioception involved?
C Weir; M Cleary; S Parks; G Dutton
1999 A method to visualise leukocytes in the retinal and choroidal circulation in vivo
P Hossain; J Liversidge; A Manivannan; P Vieara; P Sharp; J Forrester
1998 Tacrolimus in high-risk corneal and limbal transplants
C M Sloper; H S Dua; R J Powell
1997 Rapid suture management of post-keratoplasty astigmatism
A R Sarhan; M Beach; H S Dua

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.

2009 *Interleukin-1 beta induced RNase-7 expression requires MAPK but not NF-kB signalling*
I Mohammed; A M Yeung; A Abedin; A Hopkinson; M Mathew; H S Dua
2008 Human Choroidal Endothelial Cell Growth Factor signalling in Age-Related Macular Degeneration
E A Stewart; A C Browning; W M Amoaku
2007 Mutations in FRMD7, a Novel Gene, Cause X-linked Congenital

Idiopathic Nystagmus
S Thomas; F Proudlock; N Sarvananthan; E Roberts; R Trembath; M R Stratton; F L Raymond; P Tarpey; I Gottlob

2006 Amniotic membrane for ocular surface reconstruction: Donor variations and handling affect membrane constituents
A Hopkinson; R S McIntosh; P J Tighe; D K James; H S Dua.

2005 In vivo confocal microscopy: Corneal changes following retinal detachment surgery with intra-ocular silicone oil
K H Weed; A W Ferguson; J D Ellis; P S Baines

2004 The isolation and characterisation of adult human sub-macular inner choroidal endothelial cells
A Browning; W M Amoaku

2003 Characterisation of an in vitro model for studies into age related macular degeneration
R D Hamilton; A L Foss; L Leach

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

2009 Expression Paterrn of Anti-microbial peptides (AMPs) in Acanthamoeba Keratitis
A M Otri; I Mohammed; A Abedin; N Panjwani; A Hokinson; H S Dua

2008 Malignancies after Tacrolimus Therapy in the management of ocular inflammatory disease
M Mathew; D Raj; K Mohammed; A Abedin; H S Dua

2007 A 24-months follow-up of severe ocular burns with impression cytology
J-J Gicquel; R Navarre; M E Langman; M Mercie; S Milin; P-M Levillain; J-M Gombert; P Dighiero

2006 Retinal features in children with Down's syndrome
P Ji; J M Woodhouse; A L Jones; J E Morgan; P O Watts

2005 Intravitreal triamcinolone acetonide in the management of refractory uveitis
H Kolli; R W D Bell

2004 National survey of management of acquired nystagmus
I Choudhari; N Sarvananthan; I Gottlob

2003 Interactive teaching in ophthalmology
P Tesha; F Proudlock; N Sarvananthan; R McLean; J Horsley; I Gottlob

2002 The taut thickened posterior hyaloid (TTPH)
D Thomas; D A H Laidlaw

2001 Hyaluronic acid promotes the migration of corneal epithelial cells in vitro
R Amankwah; A O Powell-Richards; J A P Gomes; W M K Amoaku; H S Dua

2000 Quantitative assessment of cytokine mRNA and secreted protein in proliferative vitreoretinopathy
I A El-Ghrably; H S Dua; G M Orr; D Fischer; P J Tighe

1999 Does ethnic origin influence the incidence or severity of keratoconus?
A Pearson; B Soneji; N Sarvananthan; J Sandford-Smith

1998 Modified Sheridan Gardiner vision test with semi-transparent card
R Ahmed; H S Dua

1997 Stem cell deficiency of the corneosleral limbus : a new approach to surgical management
D Raj; H S Dua

No: 11

Patient with previously undiagnosed Autoimmune Hypophysistis (AH) presenting with bilateral recurrent cystoid macular oedema (CMO) secondary to Intermediate uveitis

Amjad, M., Sachdev, A., Kotamarthi, V.

Leighton Hospital, Crewe

Introduction:
AH is a rare destructive autoimmune disorder of pituitary gland, mainly seen in women either in post-partum period or with other auto-immune disorders, leading to its impaired functioning.

Purpose:
The aim of this case report is to demonstrate that the first and only clinical manifestations of AH could be CMO with intermediate uveitis and can present in male sex.

Methods:
55 years male caucasian presented with complaints of decreased vision and changing refraction of 12 months duration. Examination showed bilateral intermediate uveitis and CMO confirmed by OCT with no other vitreous or fundus abnormalities.

Results:
FBC, biochemical, chest X ray, autoimmune screening, Treponema Ab, Toxoplasma Ab did not show any abnormalities. Detailed history revealed that he has been suffering from worsening symptoms of polyuria, lack of energy and libido. The MRI scan of brain revealed inflammatory swelling of pituitary which was abutting optic chiasma; in addition, the auto-antibodies against pituitary were positive confirming diagnosis of AH. Automated perimetry was normal. He was treated with IV followed by oral steroids and is on hormone replacement therapy. His recurrent episodes of bilateral intermediate uveitis with CMO responded very well to both sub-tenon and Intra-vitreal triamcinolone injections on different occasions, confirmed by OCT, which improved his visual acuity as well. His AH and visual symptoms are stable for more than a year.

Conclusions:
We present an unusual case of ocular presentation of AH in a male patient previously undiagnosed with autoimmune endocrine disorder. Such a case has not been reported before.

No: 10

Human β-defensin 9, a 'functional' host defence protein

Mohammed, I., Hopkinson, A., Suleman, H., Chen, P., Otri, A.M. and Dua, H.S.

Division of Ophthalmology and Visual Sciences, University of Nottingham

PURPOSE: Host defence protein (HDP; also known as antimicrobial peptides) plays a key role in mucosal innate immunity. In our previous investigation, we have demonstrated the down regulation of the Human β-defensin 9 (HBD-9) in ocular surface (OS) health and disease. However, other groups have reported HBD-9 as a pseudogene or non-functional gene. Therefore, to further study HBD-9, we have set out following aims, a) to characterise the antimicrobial activity of HBD-9, b) to investigate the protein and mRNA expression of HBD-9 in response to the pathogen-associated molecular patterns (PAMPs), c) to identify the signalling pathways involved in TLR2 induced HBD9 expression and d) to investigate the affects of dexamethasone on HBD-9 expression.

METHODS: HBD-9 protein was expressed in *E.coli* competent strains using established methods. Antimicrobial activity of recombinant HBD-9 (rHBD-9) was tested against Gram-positive and Gram-negative bacteria. Using Immunofluorescence (IF), localisation of HBD-9 protein was described on OS tissue sections. Effect of synthetic PAMPs on HBD-9 mRNA and protein regulation was investigated using Quantitative PCR (qPCR) and IF respectively. Activation of TLR2 signalling pathway in response to Pam3CSK4 (P3C4) was studied using Western blotting. The role of TLR2 signalling pathway in HBD-9 expression was elucidated by blocking essential signalling molecules using pharmacological inhibitors and RNAi interference technology. The resultant change in HBD-9 expression was further studied by qPCR, IF and ELISA. Furthermore, the affect of dexamethasone on HBD-9 expression was also studied using a variety of above-mentioned techniques.

RESULTS: rHBD-9 has demonstrated an effective antimicrobial property against tested microbes. HBD-9 has shown high expression in all layers of OS tissue. TLR2, 3 and 5 was demonstrated to play key role in upregulation of HBD-9 at the OS. P3C4 has been shown to activate both NF-κB (nuclear factor-kB) and mitogen-activated protein kinase (MAPK) signalling pathways in a time-dependent fashion. HBD-9 mRNA and protein expression was reduced upon blocking and siRNA knockdown of both NF-κB and MAPK pathways. Dexamethasone was shown to down regulate TLR2 induced HBD-9 expression in both dose- and time-dependent manner via activation of dual specificity phosphatase-1 (DUSP-1; also known as MAPK phosphatase-1 (MKP-1)).

CONCLUSION: It is the first study that unveils the functional role of HBD-9 in host defence system. The involvement of multiple signalling pathways in HBD-9 mRNA and protein in response to TLR2 may facilitate the development of novel approaches in enhancing antimicrobial defence and healing of the eye.

Research Presentation Abstracts

In order of presentation on the day

No: 9

Variable penetrance associated with FRMD7 mutations

Thomas, S., Thomas, M.G.*, Gottlob, I.*

Ophthalmology Group, University of Leicester, UK
*The first two authors should be regarded as joint first authors

Purpose: To investigate whether the type of mutation and location of the mutation accounts for the variability of penetrance among females in FRMD7 mutant families.

Methods: Families (n=30) with mutations of the FRMD7 gene were identified based on sequence analysis. Mutations were classed based on type into non-truncating and truncating. Mutations were also subdivided based on which domain of the protein was affected. The FRMD7 protein was divided into 5 domains: FERM-N, FERM-M, FERM-C, FA and Unstructured C-terminal domain. Female obligate carriers within each pedigree were identified and classified as affected or unaffected with nystagmus based either on eye movement recordings (EyeLink II, SR Research, Toronto) or examination by an ophthalmologist. A linear mixed model was used to test the above hypothesis.

Results: Most mutations were clustered in the FERM-C domain. There was no significant difference ($p>0.05$) based on both mutation class and domain affected. Though there were some notable trends in penetrance within some families.

Conclusion: We show for the first time that FERM-C domain was affected with highest incidence of mutations. Previously, it has been described that truncating mutations were associated with lower penetrance compared to non-truncating mutations; possibly because truncating mutations were associated with a more deleterious cellular phenotype resulting is cell selection where the wild type allele is active. Our data does not conform to these findings and suggest that non-truncating mutant proteins are unlikely to exhibit a dominant negative effect.

No: 8

In vivo confocal microscopic findings in patients with limbal stem cell deficiency

Miri, A., Alomar, T., Dua, H.S.

Division of Ophthalmology and Visual Sciences, University of Nottingham

Purpose:
To describe in vivo confocal microscopy (IVCM) findings in patients with limbal stem cell deficiency (LSCD). The objective is to be able to diagnose early and late cases of limbal stem cell deficiency without the need for invasive methods such impression cytology or ocular surface biopsy.

Methods:
17 eyes of 12 consecutive patients suffering from limbal stem cell deficiency were included in this study.
A detailed examination by IVCM was performed in addition to a routine slit-lamp biomicroscopy. Size and density of corneal epithelial and conjunctival cells were measured and statistically analyzed using SPSS software.

Results:
IVCM examination of the limbus confirmed the conjunctivalisation of the cornea in all eyes and the absence of palisades of Vogt's in 14 eyes. Anatomical and morphological differences were noted between corneal and conjunctival cells. Size and density differences reached to significant levels between the normal corneal cells and the conjunctivalised cells ($P < 0.04$ and 0.05 respectively). Goblet cells were visible throughout the conjunctivalised epithelium in some but not all eyes.

Conclusion:
Laser IVCM can be used to establish diagnostic features of a defective limbus in patients manifesting clinically with LSCD. The features determined by IVCM correlate with features observed in vitro, by histological examination of impression cytology and biopsy specimen.

Corneal Biomechanical Parameters in Asymmetrical Glaucoma

Poostchi, A., Henry, E. and Vernon, S.A.

University Hospital, QMC, Nottingham

Introduction
Corneal biomechanical parameters may influence applanation tonometry. With the exception of corneal thickness, until now these have been difficult to measure in vivo.

The Reichert Ocular Response Analyzer (ORA) is a new non-contact tonometer that uses a metered air pulse to deform the cornea into a slight concavity and an electro-optical system to detect the timing of the corneal deflection from which the IOP can be determined. Additional biomechanical information can be inferred from the shape and timing of inward and outward applanation signals recorded by the machine.

Abstract

Purpose: To identify inter-eye differences in corneal biomechanical parameters as measured by a non-contact tonometry and pachymetery in patients with asymmetrical or unilateral glaucomatous visual field loss.

Methods: Records of 34 patients with glaucoma with asymmetrical visual fields were reviewed. Intraocular pressures and biomechanical parameters were recorded for each eye.

Results: Corneal biomechanical parameters were found to be lower in eyes with worse visual field loss (mean CH=10.0, CRF=10.6, CCT=548) compared to eyes with less field loss (mean CH=9.4, CRF=10.3, CCT=544). Using conventional levels of statistical significance, this was significant for both CH ($p=0.005$) and CRF ($p=0.03$). A similar trend was seen for central corneal thickness but this did not reach statistical significance ($p=0.07$).

Conclusion: Corneal hysteresis and corneal resistance factor is lower in eyes with worse field loss in asymmetrical glaucoma.

Comparative Transcriptional Profiling of Limbal Epithelial Crypt with other Ocular Surface Epithelium with Gene ST 1.0 Array

Kulkarni, B.[1], Arno, M.[2], Aldecoa-Otalora Astarloa, E.[2], Hopkinson, A.[1] and Dua, H.S. [1]

1. Division of Ophthalmology and Visual Sciences. University of Nottingham
2. Genomics Centre, King's College, London

Purpose: Anatomical and immunohistological studies of Limbal Epithelial Crypts (LEC) demonstrates its potential as a stem cell niche. We aim to characterise the transcriptional profile of the LEC in comparison with other ocular surface epithelial regions, with Gene ST 1.0 Arrays to identify its characteristics and any expression of putative stem cell marker(s).

Methods: The frozen cryostat sections of the corneoscleral epithelium from cadaver donor tissue were Laser Microdissected (LCM) with PALM® Microbeam systems followed by RNA extraction. The extracted RNA was amplified & hybridized to Human Gene ST-1 arrays. Following hybridisation quality controls, the raw data (CEL) files were normalised and back ground noise factors eliminated. Data set of 29,000 genes was uploaded on Qlucore software. Gene lists for each region was created with Multiclass and two class comparisons. Gene annotations were determined with Netaffx and biological processes with Ingenuity Pathway Analysis. Further validation was carried out with Quantitative PCR (qPCR) and immunofluorescence.

Result: 463 significant genes were found to be differentially expressed across the samples of the LEC, cornea and limbus. The uniquely expressed cellular and molecular functions in LEC were in category of Hair and skin development, DNA replication, recombination and repair. In limbus the overrepresented cellular functions were inflammatory response, amino acid metabolism. Cellular functions in cornea were Antigen presentation, cell cycle, cell proliferation and movement.

Conclusion: With comparative transcriptional profiling we had identified biological processes and gene expression patterns of each OS region with emphasis on the potential features of LEC, as a stem cell niche.

No: 7

Therapeutic limbal stem cell delivery using hydrogels

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

University of Reading

Purpose: A primary cause of corneal blindness is limbal stem cell deficiency (LSCD). The recovery of sight is often not achieved with the main treatment, limbal stem cell transplantation (LSCT). LSCT involves *ex vivo* expansion of limbal stem cells and removing this lengthy preclinical stage would be an advantage. The aim of this study is to investigate hydrogels as limbal stem cell carriers which support cell viability and differentiation.

Methods: Primary limbal epithelial cells from bovine eyes (model system) were suspended in a 0.6% calcium alginate gel for 24 hours under physiological (37°C, 5% CO_2) and ambient (18-22°C, atmospheric CO_2 levels) conditions. Cell viability was analysed utilising the Trypan blue assay that involves the incorporation of the blue Trypan dye into dead cells and the exclusion of this dye from live cells. Cells were fluorescently labelled with Cyto-dye (live cells-green) and propidium iodide (dead cells-red) to visualise their viability. Immunoblotting analyses were performed to measure changes in protein markers of differentiation (CK3) and undifferentiation (CK14).

Results: Primary epithelial cells survived well within gels, as 70-80% of extracted cells were alive. The visualisation of live and dead cells supported these data. CK14 levels were lower in cells within gels under physiological conditions than in cells within gels under ambient conditions. CK3 levels were slightly higher under physiological conditions than under ambient conditions.

Conclusions: The robust viability of primary epithelial cells, as well as their ability to differentiate within a calcium alginate hydrogel demonstrated that this substrate has potential for supporting limbal stem cells before transplantation.

No: 6

In vivo confocal Microscopy in Corneal Oedema with Histopathological correlation

Alomar, T., Lowe, J., AL-Aqaba, M.A., and Dua, H.S.

Aim: To report in vivo confocal microscopic (ivcm) features in cases of corneal oedema with correlation to corresponding histopathological changes.

Design: Observational Study

Methods: 20 patients with clinically diagnosed corneal oedema were involved in this study. 10 cases were diagnosed with Fuch's Endothelial Dystrophy (FED). All cases were examined with ivcm prior to corneal graft operation. 4 cases underwent Decemet's membrane Stripping Endothelial Keratoplasty (DSEK) and one case had Amniotic membrane transplant after removal of oedematous corneal epithelium. Tissue samples were processed for light microscopic examination which was followed by correlative analysis of Histopathological and ivcm features.

Results: Sub-epithelial fibroblasts were seen histologically and with ivcm in 7 of 15 cases with full thickness corneal sample. These cells were seen in 10 cases (50%) of the study group. In FED group no clinically detectable fibrosis could be seen preoperatively. Other ivcm features included reduced or absent sub-basal corneal nerves (100%), expanded bright keratocyte cell bodies and processes with poor nuclear visibility and large inter-keratocyte lacunae. These features were not illustrated well in Histopathological sections. In addition endothelial polymegathism with reduced cell density were seen in non-FED cases.

Conclusion: Ivcm correlates well with Histopathological examination at certain corneal layers. Still this non invasive microscopy shows details not well demonstrated in histopathological sections probably due to methods of tissue processing. Ivcm detection of sub-epithelial fibroblasts and reduced sub-basal corneal nerves needs more elaborate clinical research work.

Biomechanical properties of amniotic membrane preparations for ophthalmic surgery

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

Division of Ophthalmology and Visual Sciences, University of Nottingham

Purpose
To quantify biomechanical changes in amniotic membrane (AM) following cryopreservation and lyophilisation. These properties correlate with the suitability of different AM preparations for ophthalmic surgical use.

Methods
AM from 13 healthy elective caesarean sections was isolated, washed and cut into sections. Sections were kept fresh (FrAM), cryopreserved (CPAM) or freeze-dried (FDAM). Frozen sections were then thawed, and lyophilised AM rehydrated for testing. Uniaxial tensile property testing was carried out on 10 mm x 30 mm AM strips using a commercial materials testing machine (Stable Micro Systems, Godalming, UK) to determine the following 4 measurements at the point of failure: load (N), force-distance gradient (N/mm), work done (Nmm) and strain (ε_f). At least 60 tests were carried out per group.

Results
Multivariate ANOVA tests were used to remove outliers and to compare results. FrAM had significantly higher load (rupture strength, mean 2.13 N, SD 0.78, n = 51) and gradient (stiffness, 0.47 N/mm, SD 0.18) than FDAM (1.72 N, SD 0.66, 0.35 N/mm, SD 0.15, n = 58; p=0.008 and 0.001 respectively). CPAM was significantly stiffer than FDAM (0.43 N/mm, SD 0.16; p=0.034, n = 50). All other measurements did not show statistically significant differences between preparations.

Conclusions
Measurements of load at rupture, gradient and work done correlate with stress-strain measurements (failure stress, elasticity modulus and modulus of toughness), used in materials testing. We have shown that freeze-drying weakens AM by approximately 20%, significantly more than cryopreservation. Future work is in progress to strengthen the biomaterial for surgical use.

Differentiation status of limbal epithelial cells cultured on intact and denuded amniotic membrane before and after air-lifting

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

Stem cells and nanomaterials laboratory, Reading School of Pharmacy, University of Reading

Purpose: To investigate differences in bovine limbal epithelial cell differentiation when expanded upon intact (amniotic epithelial cells remaining) and denuded human amniotic membrane.

Methods: AM is a commonly used substrate in *ex vivo* expansion and ophthalmic surgery for corneal stem cell transplantation. *Ex vivo* expansion of the epithelial cells, in supplemented media, continued for 2 weeks followed by 1 week under 'air-lifting' conditions. Before and after air-lifting the differentiated (K3/K12 positive) and undifferentiated (K14 positive) cells were quantified by immunohistochemistry, Western blotting and quantitative PCR.

Results: Limbal epithelial cells expanded upon amniotic membrane formed 4-6 stratified layers, on both intact and denuded amniotic membrane. On denuded amniotic membrane the proportion of differentiated cells remained unaltered following air-lifting. Within cells grown on intact amniotic membrane, however, the number of differentiated cells increased significantly following air-lifting.

Conclusions: These results have important implications for both basic and clinical research. Firstly, they show that bovine limbal epithelia can be used as an alternative source of cells for basic research investigating *ex vivo* limbal stem cells expansion. Secondly, clinicians can now make an informed choice between transplanting an immature ocular surface comprised of undifferentiated cells (intact amniotic membrane before air-lifting) or a more mature ocular surface (denuded amniotic membrane).

No: 5

Toll-like receptors mediated gene expression of LEAPs at human corneal epithelium

Chen, P., Mohammed, I., Hopkinsion, A. and Dua, H.S.

Division of Ophthalmology & Visual Sciences, University of Nottingham

Purpose

An important part of the defence system for multicelluar organisms is a group of peptides with antimicrobial activity which are known as antimicrobial peptides (AMPs). Their expression is generally modulated by TLRs, transmembrane proteins responsible for the recognition of pathogens. In addition, liver expressed antimicrobial peptides (LEAPs) are considered to be AMPs due to the fact that they resemble the common structural elements and antimicrobial activity of other peptide antimicrobials. However, the expression of this type of AMP on the human ocular surface has yet to be described. The aim of this study therefore, is to explore TLR-mediated gene expression of LEAPs at the human corneal epithelium following treatment with pathogen-derived ligands.

Methods

Human SV40 transformed immortalized corneal epithelial cell line was treated with pathogen-derived ligands and changes in gene expression of LEAP-1 and -2 were determined using quantitative real-time PCR.

Results

Compared to untreated group, there was no significant change found in expression of LEAP-1 in response to stimulation of ligands ($p>0.1$). In contrast, LEAP-2 performed a notable up-regulation ($p<0.01$) following the treatment by lipopolysacchride (LPS).

Conclusions

LEAP genes are constitutively expressed at corneal epithelium but at a relatively lower level than other AMPs. The difference in expression of LEAP-1 and -2 upon treatment by pathogen-derived ligands indicates that multiple factors might be involved. Although induction of LEAPs at the corneal epithelium seems preferably sensitive to the Gram-negative bacterial infection, the exact functional characteristics of LEAPs, and the mechanism by which they are induced, remain to be defined.

No: 4

A Study Assessing the Effect of Tenotomy Procedures on Infantile Nystagmus

McLean, R., Kumar, A., Proudlock, F. and Gottlob, I.

University of Leicester

Purpose
To assess the validity of the tenotomy procedure in patients with infantile nystagmus in terms of visual acuity and nystagmus intensity.

Methods
Eye movements (500Hz) and LogMAR visual acuities were recorded from 6 patients with infantile nystagmus before and after a tenotomy procedure was performed. Eye movements and visual acuity (VA) were recorded at distance (1.2m) and near (0.4m) for 4 patients with albinism and nystagmus (1 of which had combined tenotomy/strabismus surgery) and 2 patients with idiopathic periodic alternating nystagmus (PAN). Nystagmus intensity was measured at positions from -30° to 30° eccentricity (for both 1.2m and 0.4m) at 3° intervals along the horizontal plane and the data was compared before and after (1wk, 4wks, 8wks, 12wks) surgery. Changes in intensity were viewed at null point and across all positions and compared to changes in VA. The patients' own subjective estimates of change in VA and nystagmus were also recorded.

Results
In terms of LogMAR VA the albinism patients improved by 13.8%, 17.8% and -3.5% for distance and 15.3%, 14.8% and 12.6% for near. The combination tenotomy/strabismus patient improved by 3.4% for distance and 18.8% for near. . The amount of improvement appeared to be dependent upon gaze position. Both PAN patients showed no change in VA. Variable changes were found on eye movement recordings, particularly reduction of nystagmus intensity in lateral gaze. All patients felt subjectively that their nystagmus had improved and all but one patient felt they had an improvement in VA.

Conclusion
Early reports suggest that tenotomy may improve infantile nystagmus. However, it is necessary to evaluate more patients to substantiate these findings and find which patients respond best to the procedure.

Amniotic membrane transplantation on the ocular surface: A Meta-Analysis

Suleman, H.[1], Lazutina, .E[1], Gicquel, J.J.[2], Yeung, A.M.[1], Mohammed, I.[1], Tint, N.L.[1], Desai, P.[3], Dua, H.S.[1]

[1]Division of Ophthalmology and Visual Sciences, University of Nottingham, [2]University of Poitiers, Poitiers. France, [3]Moorfields Eye Hospital, London.

Purpose: To evaluate the overall effectiveness of amniotic membrane transplantation (AMT) in ocular diseases.

Design: Systematic review and meta-analysis of randomised controlled trials.

Methods: Relevant studies were selected through an electronic search of PubMed, the Cochrane Central Register of Controlled Trials and EMBASE. The randomised controlled trials meeting the criteria were reviewed systematically by meta-analysis. Successful treatment comparing AMT to controls was measured as odds ratios, and the pooled odds ratio and 95% confidence intervals were computed according to a fixed or random effects model depending on heterogeneity. Subgroup analysis was conducted where there were four or more studies relating to a particular pathology or surgical procedure.

Results: Nine trials involving 665 patients were included in the present meta-analysis. The pooled odds ratio of a successful outcome with AMT compared to controls was 0.75 (95% confidence interval [CI] 0.35 to 1.62, $p=0.47$).

In the management of pterygium, 4 trials involving 501 patients, showed the pooled odds ratio of a successful outcome with AMT compared to conjunctival autograft as 0.33 (95% confidence interval [CI], 0.20 to 0.54, $p<0.001$).

Conclusions: Amniotic membrane transplantation is equivalent in efficacy (or possibly less so) to the other modalities of treatment presently available for ocular diseases. In the management of pterygium, amniotic membrane is associated with an inferior outcome compared to conjunctival autograft and therefore should be avoided. Clinicians need to carefully assess the advantages and disadvantages of AMT and the alternatives before considering this treatment.

Expansion of Human Mesenchymal Stem Cells on Amniotic Membrane for Use in Occular Surface Repair

Branch, M., Hopkinson, A., Jones, R. and Dua, H.S.

University of Nottingham

Current management options for limbal stem cell (LSC) deficiency and loss of the corneal epithelium (CE) include allogeneic or autologous LSC transplantation which present complications of graft rejection and immunosuppression or contralateral iatrogenic injury respectively.
Ex vivo expansion of limbal explants on amniotic membrane (AM) is a tried and tested method of restoring function to the cornea. However these cells show varying degrees of differentiation and do not proliferate extensively *in vitro* which influences the graft survival leading to unpredictable clinical outcomes.
As a carrier for the expanded cell sheet AM has many desirable properties including; promotion of CE proliferation, maintenance and stemness, protection of the newly created epithelium and dissolvability on the eye over time. Furthermore AM does not generate a noticeable immune response in the eye.

Human mesenchymal stem cells (hMSC) can be easily harvested from a range of autologous tissues; they grow well *in vivo* and as such are increasingly being investigated as a readily available tissue-substitute.

There is growing evidence to suggest hMSC are able to repopulate the corneal epithelium. Improve corneal wound healing and transdifferentiate into corneal epithelium.
This study analyses growth, interactions and phenotypic characteristics of hMSC on AM. This was achieved by analysis of MSC morphology and marker expression profile. MSC adhered to denuded AM and grew prolifically on the basement membrane. They continued to display MSC-like morphology and expressed a range of stem cell markers throughout.
This study highlights the potential for expansion of MSC on AM as a suitable approach for MSC transplantation onto the ocular surface

No: 3

Keratocytes Behave like Stem Cells

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

Division of Ophthalmology and Visual Sciences, University of Nottingham

Background
Stem cells are defined on the basis of two unique properties which are
1. there self renewal capability and 2. there potency to differentiate into other cells.
In cornea there are two regions where stem cell reside 1.Limbal Epithelium (containing limbal stem cells) and 2.Limbal Stroma (containing keratocytes).

Methods
Keratocytes were cultured from the limbal region from 3 rims (as it has been shown that keratocytes from perilimbal region has the greatest capacity for proliferation as well as the best clonogenic potential).
Cells were then cultured continuously up to 9 generations (passages) from each rim until the cells reach confluence stage. Two million cells were taken from each rim and then divided into 5 tubes each (containing roughly 4×10^5 in each tube) and flow cytometry was performed using mesenchymal (cd29 and cd105) and haematopoietic markers (cd34 and cd45) respectively.

Results
At early passages (P0-P2), keratocytes expressed both haematopoietic and mesenchymal markers but at the later passages, the expression of haematopoietic markers was highly down regulated. On the other hand, expression of mesenchymal markers was retained nearly to 100%.

Conclusion
This is the first study that demonstrates the stem-like characteristics of the corneal keratocytes. In particular, we have shown both haematopoietic and mesenchymal characteristics, suggesting that these cells can be used in various ocular and non ocular disorders. Hitherto, further study is highly warranted for validation of our key finding by means of protein and mRNA studies.

No: 2

Comparative study of analgesic effectiveness of three different topical anaesthetics for intravitreal injection of ranibizumab

Moodie, J., Trivedi, D., Puri, P.

Royal Derby Hospital, Derby

Purpose
Compare the analgesic effectiveness of topical proxymetacaine, tetracaine, and oxybuprocaine for intravitreal injection of ranibizumab.

Method
361 patients undergoing intravitreal injection of ranibizumab over a 3 month period were randomized to receive one of either topical proxymetacaine, tetracaine, or oxybuprocaine. Analgesic effectiveness was recorded by the patient on a pain scale (1 = no pain, 10 = most severe pain). Patients were asked to grade their pain on instillation of their eye drops pre-injection, during injection, and 24 hours post-injection. An average pain score for the entire procedure was also calculated. Analgesic effectiveness of the 3 anaesthetic drops was compared using the ANOVA test.

Results
The mean pain scores are summarized below for proxmetacaine, tetracaine, and oxybuprocaine respectively:

Pre-injection: 1.29 (SD 0.77); 1.54 (SD 1.17); 1.25 (SD 0.68); ANOVA p=0.027
During injection: 1.65 (SD 0.97); 1.64 (SD 1.35); 1.72 (SD 1.32); ANOVA p=0.871
Post-injection: 1.70 (SD 1.31); 1.91 (SD 1.49); 1.92 (SD 1.16); ANOVA p=0.787
Entire procedure: 1.51 (SD 0.68); 1.64 (SD 0.92); 1.51 (SD 1.08); ANOVA p=0.292

Conclusion
Patients found tetracaine drops to be slightly more painful pre-injection compared to proxymetacaine or oxybuprocaine. When considering the overall pain experienced for the entire procedure there appeared to be no significant difference between the 3 topical anaesthetics. The type of topical anaesthetic used could therefore be chosen on other factors such as the degree of corneal toxicity, cost, or surgeon preference.

Inadvertent Total Loss of Descemet's Membrane in a Deep Anterior Lamellar Keratoplasty-an Unusual Complication

Ramamurthi, S., Obi, E., Mantry, S., Ramaesh, K.

Gartnavel General Hospital, Glasgow

Purpose: To report an unusual intra-operative complication in a case of Deep anterior lamellar keratoplasty and a novel observation of graft recovery.

Method: Case Report
A 43 year male underwent DALK for corneal scarring secondary to herpetic keratitis. A big bubble technique was used to prepare the host bed (7.5mm).The donor graft, 7.75mm was prepared by removing the Descemet's membrane(DM) and the donor was placed onto the host bed .Whilst doing the initial stay suture, the globe was pulled inadvertently and a Descemet's membrane rupture of almost 360 degrees occured. Since there was no graft material available, the procedure was completed with the graft secured in place, without the DM or the endothelium, with a 10.0 nylon interrupted suture with a view to re-grafting at a later date.

Result: Post-operatively the graft was oedematous for three months and visual acuity was hand movements. A re-graft was considered. At four months from the initial graft his uncorrected visual acuity had improved to 6/36 and the graft oedema began to resolve gradually. The BCVA at 6 months post-operatively was 6/6 and the graft oedema resolved completely. The confocal microscopy showed endothelial cells on the graft which was devoid of the Descemet's membrane.

Conclusion: This is a unique case and we believe the resolution of oedema is due to the migration of the endothelium from the host to the donor bed.

Thermoreversible PLGA-PEG-PLGA Copolymer Hydrogel for Ocular Surface Reconstruction

Tint, N.L., Dhillon, A., Shakesheff, K.M., Dua, H.S. and Rose, F.R.

University of Nottingham

Background/Aims: To fabricate novel blends of poly(lactic-co-glycolic acid) (PLGA), Poly-ethylene Glycol (PEG) copolymer to serve as a biomimetic bandage for ocular surface reconstruction. This will be fully characterised using rheology as well as demonstrating *in vitro* biocompatibility.

Methods: The PLGA–PEG–PLGA triblock copolymer was synthesised via ring-opening polymerization using stannous octate in argon atmosphere. Various ratios of PLGA and PEG were used. 25%, 30%, 35% and 40% hydrogel solutions were made and characterized using a rheometer as well NMR (Nuclear Magnetic Resonance) and GPC (Gel Permeation Chromatography). *In vitro* biocompatibility studies were undertaken using viability and cytoxicity assays.

Results: PLGA-PEG-PLGA triblock copolymer was successfully synthesised as confirmed by NMR and GPC. Sol-gel transition can be altered by changing molecular weight of PEG. PEG 1000 led to down shifting of the transition temperature whilst PEG 1500 increased the temperature. Variations in batches were seen, however, desired sol-gel temperature was achieved by blending. Cytotoxicity testing did not reveal adverse affect of the hydrogel and its breakdown products.

Conclusion: Successful synthesis of PLGA-PEG-PLGA copolymer hydrogel was achieved. Rheology showed that the desired sol-gel transition temperature can be achieved, by blending. Furthermore, the hydrogel demonstrated *in vitro* biocompatibility with human corneal keratocytes. This hydrogel may have a role as a synthetic biodegradable corneal bandage as well as potential as a mode a drug delivery to the ocular surface.

No: 1

Agreement between classification by trained optometrists and Heidelberg Retinal Tomography in a normal elderly population

Poostchi, A., Hawker, M., Crabb, D.P.*, Vernon, S.A.

*University Hospital, QMC, Nottingham,*Optometry and Visual Science, City University London.*

***Introduction**: The Heidelberg Retinal Tomograph is a commercially available confocol scanning laser ophthalmoscope that can be used to image the optic nerve head. The device contains a number of data analysis tools including cup shape measures, discriminant functions and the Moorfields Regression Analysis (MRA). More recently an automated shape analysis tool which calculates a Glaucoma Probability Score (GPS) and does not require manual placement of a disc contour line has been developed.*

Purpose: To determine the agreement between classification by trained optometrists, MRA and GPS in a normal elderly population.

Participants: 1096 eyes of the first normal (defined with visual fields and intraocular pressures) 548 subjects (mean age 72 years, range 65 - 89 years) from The Bridlington Eye Assessment Project.

Methods: Patients underwent optic nerve imaging using an HRT 2. Scan data was imported into a later version of the device for calculation of GPS. 458 subjects with a HRT mean pixel height standard deviation (MPHSD) of less than 68 um were included in the analysis.

Results: Agreement between classification methods was poor with best K coefficient seen between clinical evaluation and MRA2 (K = 0.22, 95% CI 0.06 - 0.38).

When borderline cases were classified as abnormal, the specificities of GPS, Moorfields Regression Analysis 2 (MRA2) and Clinical evaluation were 78 %, 83% and 95% respectively,. This rose to 84% (95% CI, 81 - 88%) and 93% (95% CI, 91 - 96%) for GPS and MRA when borderline cases were re-classified as normal.

MPHSD and Cup-Disc ratio were significant covariates ($p<0.001$) within a generalised linear model with the GPS score as the dependent variable

Of the 430 eyes considered to have a normal optic nerve head on clinical evaluation, 22% were classified as abnormal and 47% as borderline or abnormal by GPS.

Conclusion: When used in isolation, GPS is less specific than MRA2 or fundoscopy by trained optometrists when screening a population of functionally normal elderly individuals. MPHSD may be a confounder of GPS score.

Tissue engineering of the ocular surface using plastically compressed collagen gels

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

University of Reading

Purpose: To explore the use of plastically compressed collagen gels as scaffolds for tissue engineering of the ocular surface

Methods: LECs were isolated from bovine corneas (limbus) and seeded onto either conventional uncompressed or novel compressed collagen gels and grown in culture. The growth of limbal epithelial cells was compared, microscopically, between novel compressed collagen gel and traditional uncompressed collagen gel.

Results: Scanning electron microscopy (SEM) results showed that fibres within the uncompressed gel were loose and irregularly ordered while the fibres within the compressed gel were densely packed and more evenly arranged. Transmission electron microscopy (TEM) results showed the compressed scaffold to contain collagen fibres of regular diameter and similar orientation resembling collagen fibres within the normal cornea. SEM showed the expanded LECs, to have a heterogeneous morphology upon uncompressed gels, and a smooth and homogeneous morphology on the compressed gels. TEM and light microscopy also showed that cell-cell and cell-matrix attachment, stratification and cell density were superior in LECs expanded upon compressed collagen gels.

Conclusion: This study demonstrated that the compression of collagen gels influenced the morphology of subsequently expanded limbal epithelial cells.

Architecture and Distribution of Human Corneal Nerves

Al-Aqaba, M.A.[1], Fares, U.[1], Suleman, H.[1], Lowe, J.[2], Dua, H.S.[1]

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

Purpose: To comprehensively study the gross anatomy of human corneal innervation.

Methods: Twenty one specimen including 12 normal human corneas from 7 deceased patients, two eye bank corneo-scleral buttons, two eye bank corneo-scleral rims and 5 postsurgical specimens from 3 patients with keratoconus were studied. Corneal whole mounts were stained for cholinesterase enzyme using the Karnovsky & Roots direct coloring thiocholine modification of Acetylcholinestrase (AchE) technique.

Results: Approximately 44 thick nerve bundles were found to enter the human cornea in a relatively equal distribution round the limbus and move randomly toward the central cornea. At the mid-peripheral zone, anterior stromal nerves showed a characteristic budding and branching pattern. After passing through Bowman's zone they were noted to terminate into bulb like thickenings from which multiple sub-basal nerves arose. The perforation sites were predominantly located in the mid-peripheral cornea. The orientation of sub-basal nerves was mainly vertical at their origin from the perforation sites. Nerves from all directions converged toward the infero-central cornea to form a characteristic clockwise whorl pattern.

Conclusions: This study provides a comprehensive account of the architecture and distribution of nerves in the human cornea. It reconciles some of the existing information obtained from other modalities of investigation and identifies some novel features which provide a more complete picture of corneal innervation.

Research Presentation Poster Abstracts

Poster Exhibition is at the side of the Conference Hall

Phacoemulsification and toric intraocular lens insertion for correction of post penetrating keratoplasty astigmatism

Murjaneh, S., Smith, G.T.

The Great Western Hospital, Swindon

Purpose
Residual post penetrating keratoplasty (PKP) astigmatism compromises visual rehabilitation and can be challenging. We aim to assess and present the visual and refractive outcomes in our patients with post PKP astigmatism, who had cataract extraction with foldable toric posterior chamber intraocular lens (tIOL). This is a new surgical method to manage post PKP astigmatism.

Method
Retrospective non-comparative case notes review. Routine phacoemulsification was performed and a hydrophilic acrylic co-polymer toric foldable Rayner T623 lens injected into the capsular bag.

Results
Seven consecutive eyes, of six patients, who had clear corneal grafts and visually significant cataract underwent cataract extraction with tIOL between 2007 and 2008. Three had previously undergone astigmatic keratotomy. Median follow up was 11 months (SD: 4.8). The grafts remained clear with minimal change in CCT. There was a marked increase in both UCVA and SCVA post operatively. Post-operative refraction showed a mean reduction of cylindrical power of 3.1Dioptre (D) (SD: 0.78 D), on autorefaction. The lens remained properly aligned in all patients.

Conclusion
This small retrospective, non-comparative study, demonstrates that the use of a tIOL is a safe and effective procedure to improve the refractive and visual outcomes in patients with cataract, astigmatism and a clear corneal graft.

Study of analgesic effectiveness of topical drop anaesthesia for intravitreal injection of ranibizumab

Puri, P., Trivedi, D., Moodie, J.

Derby Royal Hospital, Derby

Purpose
Establish the analgesic effectiveness of topical drop anaesthesia for intravitreal injection of ranibizumab.

Method
361 patients undergoing intravitreal injection of ranibizumab over a 3 month period received topical drop anaesthesia for their injection (proxymetacaine, tetracaine, or oxybuprocaine used). Analgesic effectiveness was recorded by the patient on a pain scale (1 = no pain, 10 = most severe pain). Patients were asked to grade their pain on instillation of their eye drops pre-injection, during injection, and 24 hours post-injection. An average pain score for the entire procedure was also calculated.

Results
Mean pain scores were as follows:

Pre-injection = 1.39 (SD=0.94, range 1-8)
During injection = 1.67 (SD=1.23, range 1-9)
24 hour post injection = 1.79 (SD=1.36, range 1-8)
Entire procedure = 1.57 (SD=1.15, range 1-9)

Conclusion
Patients found intravitreal injection of ranibizumab under topical drops anaesthesia to be a well tolerated procedure. Pain scores using topical drops alone compare very favourably to those quoted in the literature for the same procedure under subconjunctival or peribulbar anaesthesia.

Infectious keratitis profile in Nottinghamshire

Otri, A.M., Maharajan, S., Dua, H.S.

Queen's Medical Centre, Nottingham

Purpose: To identify the predisposing factors, the clinical manifestation, the microbiological diagnosis, and the treatment profile of infectious keratitis in a population based in Nottinghamshire.

Methods: A prospective study was undertaken of patients presenting with infectious corneal ulcers under the care of two consultants at the eye department of Queens Medical Centre, Nottingham, UK, between October 2007 and October 2009.

Results: A total of 75 patients presented with infectious corneal ulcers with a mean age of 52 years and male predominance (56%)
Previous ocular surgery and contact lens wear were the main predisposing factors (n= 22, 29% and n= 20, 26%, respectively)
Corneal scrape were taken from 68 patients (91%). Of these, 33 patients grew positive cultures (48%). Staphylococcus aureus was the most isolated organism (n= 8, 24%), Interestingly, Acanthamoeba was the next most common cause of infectious keratitis (n= 7, 21%). There was an absolute correlation between the contact lens wear and Acanthamoeba keratitis. Fortified eye drops of cefuroxime and gentamicin or monotherapy of ciprofloxacin were the major treatment for all the cases. Corticosteroids were administered shortly after the signs of clinical response in severe cases of inflammation and infection (n= 44, 58%)

Conclusions: Previous ocular surgery and contact lens wear are the most common predisposing factors of corneal infection in our study
Gram positive cocci and Acanthamoeba are very common causes of infection

Adherence to RCOphth guidelines in monitoring patients on hydroxychloroquine by Rheumatologists

Sandhu, J., Joseph, A., Packham, J.

University Hospital of North Staffordshire

Purpose
Adherence to RCOphth guidelines in monitoring patients on hydroxychloroquine by rheumatologists is reported to be variable nationally. An audit study to evaluate the local rheumatologist's adherence to RCOphth guidelines.

Method
Medical and electronic records of patients on hydroxychloroquine attending rheumatology clinics over a 2 month period were reviewed. Data collected: patient demographics, weight, height, dosage, liver, renal functions. At baseline, and at follow-up, documentation of visual enquiry, visual impairment or eye disease, near visual acuity (NVA) and referral to an optometrist or ophthalmologist were recorded. Treatment dosage was calculated using ideal body weight (IBW) where possible, otherwise actual body weight (ABW) was used.

Results
77 (12%) patients were identified on hydroxychloroquine. Medical notes were reviewed in 67 (87.0%), and electronic letters 10 (13.0%) patients.
20 (26.0%) patients were prescribed above the recommended dose (6.5mg/kg/day), range 6.51 to 8.79mg/kg/day, (IBW n = 45, ABW n = 21, weights unobtainable n = 11). No patients were found to have concomitant retinal disease.
Baseline screening: 47 (61%) patients had enquiry of visual impairment and none had NVA assessment. All patients had renal and liver functions assessed.
Monitoring: All patients were seen annually. Visual enquiry was present in 22 patients and NVA in 1. Three patients presented with new visual symptoms, and were referred to ophthalmology.

Conclusion
Adherence to RCOphth guidelines is variable. Proposals include extended role of nurses with training to undertake NVA assessment, use IBM calculators for dosage calculation and visual impairment assessment by an optometrist, before ophthalmology referral.

Vitreous length as a predictor of refractive outcome after penetrating keratoplasty for keratoconus

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

Department of Ophthalmology, Queen's Medical Centre, Nottingham

Purpose: To determine whether vitreous cavity length (VCL) is a better predictor than axial length (AL) in the refractive outcome of patients with keratoconus after penetrating keratoplasty.

Methods: A retrospective analysis of 31 eyes (26 keratoconus patients) underwent penetrating keratoplasty from 1995 to 2002. Nine eyes had same size donor trephine, 18 eyes had 0.25mm oversize and 4 eyes had 0.5 mm oversize donor trephine. The postoperative spherical equivalent (SE) was then analyzed and was correlated to donor button size, axial and vitreous length.

Results: The mean spherical equivalent (MSE) for oversized graft was – 6.50 D $\pm$ 7.83 range (-17.5 D to -0.25 D) and the MSE for same sized graft was -1.24 D $\pm$ 3.74 range (-8.00 D to + 4.0 D).
Undersizing the donor button in keratoconus patients with increased vitreous length helped in reducing the myopic error.

Conclusions: Vitreous cavity length measurement gives a better prediction for post operative MSE than axial length measurement in deciding upon graft host size disparity. In patients with increased VCL, undersizing the donor button helps in reducing postoperative myopia. We recommend VCL measurement as part of the routine workup in all keratoconus patients undergoing corneal transplants.

A study to establish the ocular and systemic safety of simultaneous bilateral intravitreal ranibizumab injections for wet AMD

Trivedi, D., Moodie, J., Puri, P.

Derby Royal Hospital, Derby

Purpose
Establish the ocular and systemic safety of simultaneous bilateral intravitreal ranibizumab injection for wet AMD.

Method
Retrospective case series of 42 patients receiving simultaneous bilateral intravitreal ranibizumab for wet AMD from September 2008 to September 2009. Data collected included the number of intravitreal injections, patient co-morbidities, and the occurrence of an attendance to a health care professional (GP, HES, A&E department) within 4 weeks of injection.

Results
A total of 168 injections (84 simultaneous bilateral intravitreal injections) were administered to 43 patients each receiving a mean of 2 bilateral injections. Patient co-morbidities included hypertension (22), diabetes mellitus (7), cardiovascular disease (8), respiratory disease (7), hypercholesterolaemia (6), glaucoma (3), and dry eyes (2). There were 8 GP and one A&E attendance within the 4 week follow up period. Presenting complaints included shortness of breath (2), limb pain (2), skin lesions (1), rash (1), altered bowel habit (1), neuralgia (1), and fractured neck of femur (1). Of the complaints, two are recognised adverse events associated with intravitreal ranibizumab (acute bronchitis, and pain in extremities). The occurrence of these side effects was not significantly different from rates quoted in the literature from performing unilateral injections.

Conclusion
Simultaneous bilateral intravitreal ranibizumab injections appear to be safe and well tolerated. Recognised adverse events do not appear more common compared to unilateral injections. There have been no arterial thromboembolic events following injection in this study group. Aseptic technique using new instruments and vial for each eye minimizes the risk of ocular infection.

Childhood unilateral leucocoria in amateur photography

Fincham, G.S., Keightley, S.J., Sandy, C.J., Morsman, .CD.

Addenbrooke's Hospital, Cambridge

Purpose
To report the clinical findings of children presenting with unilateral leucocoria noticed on amateur home photographs.

Methods
A prospective observational case series of all children presenting to an ophthalmology outpatients department over 12 months, following concern raised by unilateral leucocoria seen on family photographs. All patients underwent full ophthalmic and orthoptic assessment. A structured questionnaire was sent to parents and written consent obtained.

Results
8 children were identified, 2 male and 6 female. Average age was 18.8 months (range 5 months to 3.1 years). 6 cases reported leucocoria to the left eye, 1 to the right and 1 alternating; in 6 cases the leucocoria had been reproducible on previous photographs. All photographs demonstrating the leucocoria were taken with indoor flash photography at a distance of 1 to 3 meters. 5 cases had no detectable abnormality on examination. 1 case was diagnosed to have hypermetropic anisometropia with amblypoia and 2 cases had esotropia. 1 of the esotropia cases was previously known to the department, and was undergoing occlusive therapy for amblyopia. Parents reported concern about the abnormal photographs was raised either through friends and family, television, press or radio reports.

Conclusions
Childhood leucocoria is reported to occur in normal eyes following amateur off-axis flash photography. However, potential sight-threatening and life-threatening conditions need to be excluded with a full ophthalmic investigation before parents can be reassured. We report an appreciable incidence of treatable amblyogenic conditions that have presented following parental concern over abnormal family photographs.

High resolution spatial and temporal expression profile of FRMD7 in neuronal tissue provides clues for pathogenesis and treatment

Thomas, M.G.[1], Araki, M.[2], Gottlob, I.[1]

[1]Ophthalmology Group, University of Leicester
[2] Department of Developmental Neurobiology, Nara University, Japan

Purpose: To investigate the spatial and temporal expression profile of FRMD7 in developing human and murine brain.

Methods: Human embryonic and fetal tissue sections were obtained from HDBR (Newcastle) at embryonic stages CS13(~28dpc) to CS23(~56dpc) and fetal stages 9wpc to 15wpc. Similar stages were also used in murine tissue. For ISH studies, sense and antisense probes were generated by subcloning PCR products (exon 9-12; NM_194277.2) into pGEM-T Easy (Promega). Anti-FRMD7 antibodies (1:250, Atlas Antibodies) were used for IHC studies. Primary culture technique was used to culture PN1 rat cerebellum followed by immunofluorescence (1:800).

Results: At stages CS15, CS16 and CS19 strong hybridisation signals were seen from the trigeminal ganglion and cerebral cortex. Both medial and lateral ganglionic eminences (MGE and LGE) showed strong FRMD7 mRNA expression at CS20. At CS22 FRMD7 mRNA expression was seen at the ventricular (VZ) and subventricular zones and the cortical plate. Interestingly, at CS23 there was strong expression in the VZ of the cerebellar peduncle as well as perihypoglossal complex at the level of the fourth ventricle. IHC results show a similar pattern with regards to the FRMD7 protein expression. Moreover, primary culture of the cerebellum showed strong FRMD7 expression along the axons and dendrites of neuroblastic cells (Purkinje cells) with no expression in glial cells.

Conclusion: This study highlights for the first time specific oculomotor centres likely to be involved in IIN. Perihypoglossal and Purkinje cell involvement supports the "leaky neural integrator" theory. Trigeminal ganglion involvement may suggest how disruption of the putative sensory signal (afferents) could be beneficial. Furthermore, MGE, LGE and VZ are the source of GABAergic and glutamatergic neurons destined for the cortical anlage. This may help explain why treatment with gabapentin and memantine are helpful in these patients.