Save Sight Institute Research Report 2003

Introduction

The research groups within the Save Sight Institute approach the problems of blindness from clinical, laboratory and epidemiological perspectives. Integrating with those exploring digital media, education and service, the groups work in close collaboration. They are listed alphabetically.

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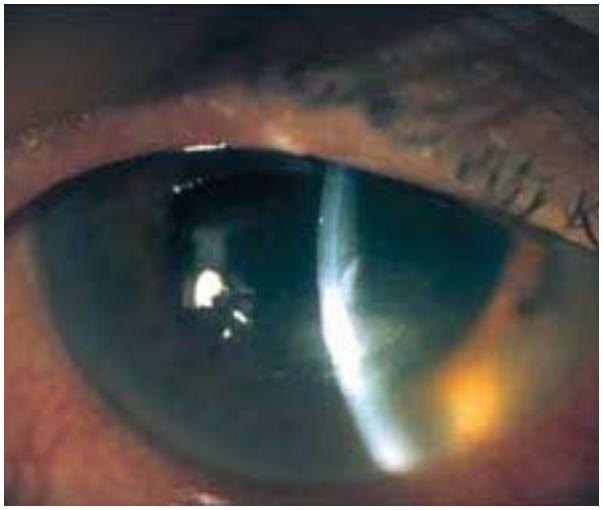


Herpes simplex stromal keratitis

Corneal Research Group

Corneal disease research in the Institute investigates the pathogenesis of the major blinding bacterial and viral corneal infections and aims to improve the outcome of corneal transplants through development of corneal storage techniques and refinement of corneal surgery.

Group Members

Kathy McClellan, MBBS PhD FRANZCO FRACS, *Unit Head*
Con Petsoglou, MBBS, MMed (Clin Epi) FRANZCO FRACS, *Lecturer*
Raj Devasahayam, BAppSc, *Eye Bank Laboratory Manager*

Research Activity

A transparent cornea makes an important contribution to eyesight by allowing light to reach the retina and providing refractive power for focusing of images. Although the cornea and outer eye have a variety of well-developed protective mechanisms, the cornea remains vulnerable to infection. Blinding corneal disease may also be caused by developmental anomalies that result in progressive corneal opacity and distortion from birth.

Herpes simplex virus (HSV) infection causes corneal scarring and loss of vision; we are investigating its distribution in trigeminal ganglia and cornea as well as its role in suppurative keratitis.

We have determined the antibiotic susceptibilities of common ocular isolates. This information allows effective and rational antibiotic therapy of corneal infection.

Finding methods of corneal storage that extend the life of corneal transplants is the focus of research in the Eye Bank Laboratories.

Clinical research in corneal disease is developing better techniques of lamellar or partial thickness corneal grafting and refining corneal transplantation in infants and children.

Projects in 2003

1. HSV distribution in human ganglia and cornea, genome specificity in cornea and lid disease, prevalence in suppurative keratitis, role in corneal graft failure without rejection and detection in iridocorneal endothelial syndrome are all being researched.

2. Antibiotic susceptibilities of corneal isolates and conjunctival flora have been determined in 125 organisms isolated from 80 patients presenting to the Sydney Eye Hospital Emergency Department during the past 12 months.

3. Optimisation of the organ culture method of corneal storage and investigation of corneal metabolism during storage is in progress.

4. Infectious keratitis is being studied in 2 groups of patients - contact lens wearers and the elderly to determine risk factors, common pathogens and outcome with aim of better preventing and managing this condition.

5. Lamellar keratoplasty for management of recurrent pterygium.

6. Corneal transplantation in infants and children. This study was the first to demonstrate graft survival beyond 14 years amongst this group of young recipients.

Digital Media Research Group

Research in this group is focused on exploiting the potential of the digital media, information technology (IT) and the communications revolution to –

•Create and implement state of the art outreach ophthalmic services for remote and rural communities.

•Develop cutting edge ophthalmic teaching services to provide excellence in education of future eye doctors.

•Empower the community through the internet by providing interactive access to information about eye care and lifestyle choices which can help prevent eye disease.

Group Members

Frank Billson, FRACO FRACS FAC, *Unit Head*
I-van Ho, *Postgraduate student*
Larry Yee, *Postgraduate student*

Research Activities

Teleophthalmology Initiative: The challenge of providing specialist eye care services outside the metropolitan centres of Australia is a significant one. This group is working to develop more effective ways of screening for eye disease in remote and rural areas.

Virtual Teaching Initiatives: The unit is researching initiatives to provide distance learning services and developing innovative ways for students to have clinical trial experience in a virtual arena before engaging in patient practice.

Interactive Community Services: As digital media increasingly become a delivery tool for community information and access to services, this group is focusing on improving ways of delivering Sight Saving Information to the community and developing simple interactive eye services to be accessed via e-media.

Projects in 2003

Virtual Ophthalmology Clinic

2003 saw the completion of further education trials using the unit's virtual ophthalmology program which allows medical students to gain skills in history taking by making use of "virtual" clinical patients. The histories in the program's database, transcribed from real patient interviews and presented by interactive virtual actors, are programmed to reflect symptoms associated with common eye diseases and complaints.

The program mimics real interactions with patients in that students control the order in which they ask questions and gather information to make a diagnosis prior to an examination. The program emails the diagnosis to the student's supervisors before allowing the student to continue the examination and higher levels of investigation. The program's strength is that it allows the student to develop

skills in interviewing and forming a diagnosis before practising on real patients. Its success as a teaching and self-development tool highlights the direction of future digital and distance learning initiatives.

The program is now deliverable via the internet. In 2003 the interface was partly redesigned for improved navigability and its networking capabilities were fine-tuned to enhance the program' s utility across a wider range of environments. It was also envisaged that it be included in the course content for the Graduate Diploma and Masters Degree in Ophthalmic Science, a distance and onsite learning collaboration between the universities of Sydney and Otago, which will begin in 2004.

Tele-ophthalmic diabetic eye screening and treatment program

This program reported in our Research Report for 2002 continued in 2003 and its post-graduate PhD student, Dr I-van Ho, provided further information to assist planning of rural outreach services for the coming years.

The program continues now in the Katherine region, an area roughly the size of Tasmania. The program of screening and recording digital images of the retina to provide information about diabetic retinopathy is now carried out by an ophthalmically trained Aboriginal health worker , Dot Butler . Recording clinical data with an additional non-mydriatic fundus camera and archiving the data generated, together with the tele-ophthalmic capabilities has meant a much better integrated service with the Royal Darwin Hospital.

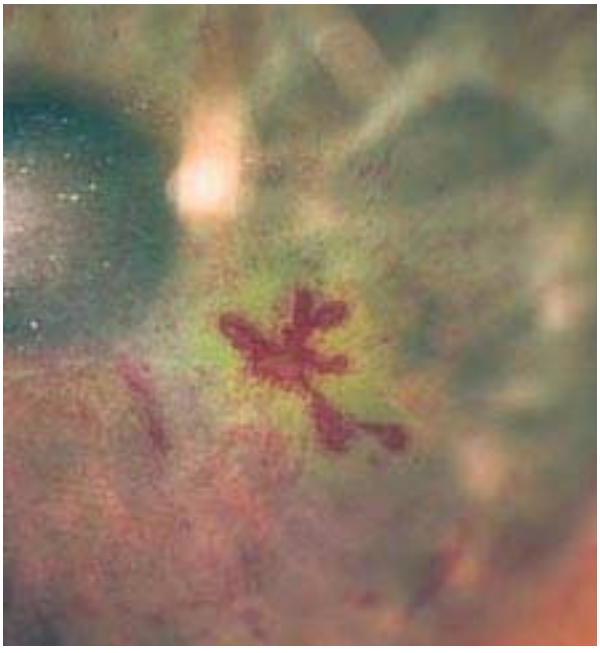
Last year we reported that the mobile ophthalmic team covered over 20,000 km, providing diabetic eye screening services in 19 out of 32 Top End rural and Aboriginal communities. It resulted in an improvement in screening coverage rates from 44% to 76% across the population. This figure has been maintained and all 32 communities have now been screened. The data has been further analysed with advice from actuarial economists; its cost-effectiveness is demonstrated by the fact that it is calculated that the service halved the costs of that anticipated if professional ophthalmologists and optometrists alone conducted the program.

Currently, a similar study is planned for the Macquarie Area Health region.

Dr Larry Yee will be doing a Masters of Medicine by thesis in this area, looking at conducting a survey of the Aboriginal population and the effectiveness of education with regard to diabetes and diabetic retinopathy.

A fully equipped facility has been developed in the Dubbo Aboriginal Medical Co-Operative Centre. A skill-transfer program will continue and the data generated will form an important opportunity in the development of a database for analysis, working with the Aboriginal Centre to develop strategies that will improve access to and understanding of eye health among Aboriginal people. It is hoped to integrate the service, with Aboriginal agreement, with the Dubbo Rural Clinical School.

This facility will be opened by the Minister for Health & Ageing, The Hon. Tony Abbott MP, on August 18th 2004.



Dendritic ulcer

Electrophysiology and Glaucoma Research Group

Group Members

John Grigg, MBBS, FRANZCO, FRACS
Alex Klistorner, Bmed, PhD
Stuart Graham, MBBS, MS, FRANZCO, FRACS
Frank Billson, FRANZCO, FRACS
Alessandra Martins, MBBS, *Postgraduate student*
Clare Fraser, MBBS, *Postgraduate student*
Chandra Balachandran, MBBS, *Postgraduate student*
Lisa Feldman, Dip Nursing, *Electrophysiology Technician*
Prya Narayan, *Electrophysiology Technician*
Asya Klistorner, *Electrophysiology Technician*

Research Activity

The electrophysiology section continues to provide clinical testing as well as research into new techniques for electrodiagnosis, especially for the detection of glaucoma. The department will soon be acquiring a new electrodiagnostic capability which will replace all our old standard testing equipment with state of the art technology for ERG/EOG/VEP recording. The Espion Diagnosys System comes programmed with the ISCEV standards as well as allowing modifications for research. It also has a hand-held mini ganzfeld which is useful for testing children. It will also enable testing of children without the need for sedation.

Projects in 2003

Development of Objective Perimetry – the Multifocal Visual Evoked Potential
Klistorner, Graham, Grigg, Billson.

In 2001 ObjectiVision Pty Ltd was set up as a collaboration between investigators at the Save Sight Institute and the Sydney University Business Liason Office to develop new techniques in vision testing. In 2002 the AccuMap V1.3 objective perimeter was launched as a new test for glaucoma that relied not upon the patient' s subjective responses but on recording the tiny electrical signals generated in the brain, called visual evoked potentials, when the patient was viewing a stimulus on a computer screen. The initial AccuMap won 2 Australian design awards in 2002.

The AccuMap then was redesigned with a major software upgrade using the OPERAV2.0 platform. This was developed to include new noise detection parameters and make the interface much more user-friendly . The system received FDA approval late 2003, and was launched in the USA at the American Academy of Ophthalmology . Heidelberg Technologies have been contracted as US distributors.

At present there are 5 US trial sites involved in the multicenter AccuMap Early Glaucoma Detection Study, with Dr Alex Klistorner and Dr Stuart Graham at the Save Sight being co-ordinators. The results of this study will help confirm the effectiveness of the technique compared to conventional methods of detecting glaucoma. Dr John Grigg and Prof Frank Billson are principle investigators.

We have recently been awarded an NHMRC Development Grant for the extension of the technique to use virtual reality goggles as a means of presenting the stimulus. This will have the potential advantage of making the test portable, and standardising the test distance.

Development of a Blue/Yellow Multifocal Visual Evoked Potential
Martins, Balachandran

This research investigates the use of blue-yellow stimuli for presenting the multifocal Visual Evoked Potential (mVEP) in an attempt to detect glaucoma at an earlier stage. The rationale for this is that in subjective perimetry blue/yellow testing has been shown to be more effective at detecting early disease. To date, the mVEP technique has used a black and white stimulus to demonstrate abnormal visual field defects in glaucoma cases. This research project has now developed an optimal design which maximises the mVEP amplitudes for the stimulus. The blue/yellow alternating checkerboard with isoluminantly matched checks has now been incorporated in the AccuMap system. This work was supported by funding from an ORIA grant in 2004.

The test has been applied to normal subjects in order to create a normal blue-yellow database.

Currently, the technique is being applied to established glaucoma patients and high risk suspects for glaucoma to establish its sensitivity in comparison with other diagnostic techniques such as Humphrey Visual Fields-the gold standard, black-white mVEP , Short-W ave Automated Perimetry and Optical Coherence Tomography.

Multifocal Visual Evoked Potentials in Optic Neuritis
Fraser, Grigg, Garrick.

Multiple Sclerosis (MS) can damage the optic nerve leading to vision loss, this can be the first presentation of MS in 30% of patients. The resulting optic neuritis causes the loss of the protective layer surrounding the nerve fibres. Consequently there is a disruption in the electrical signal transmitted from the eye to the brain. V isual Evoked Potentials (VEPs) record the electrical responses in the brain that represent the transmission of this visual information from the eye.

Current VEP recordings only measure these responses from the central visual field and cannot differentiate non-specific exacerbations of optic neuritis from true recurrences. A pilot study conducted at the Save Sight Institute has shown that the multifocal VEP (mVEP), measured by Accumap, can localise optic neuritis abnormalities to a specific visual field area and thus potentially demonstrate exacerbations of existing lesions versus new demyelination.

We are continuing the testing of the Accumap mVEP system of objective perimetry to detect changes associated with optic neuritis. The Accumap will provide an objective measure of a patient's visual field, as well as information on the axonal function between the retina and the occipital visual cortex. We hope to use the Accumap to detect subtle and more peripheral vision changes than conventional VEP, and to monitor recovery of nerve function. By studying the changes in mVEPs seen in acute optic neuritis and then following these changes over time we hope to develop the mVEP as useful tool for monitoring the effects of the new treatments for MS on recovery of visual function. Old lesions can be identified, but a chronic inflammatory change (potentially reversible) in the optic nerve may be also be identified if it can be seen to resolve with new forms of treatment.

We also hope to detect sub-clinical remissions or new lesions within the visual pathways. This may allow more patients earlier access to interferon therapy. Furthermore the mVEP could be a sensitive tool to detect remyelination, and if this could be achieved, help evaluate future therapies.

Lens Research Group

Research in this group is directed at identifying molecules and mechanisms that govern the behaviour of lens cells in health and disease. Our studies have identified molecules that play key roles in both normal and pathological lens development. Currently we are working to gain a better understanding of how these molecules are regulated in the eye. This is fundamental to identifying new therapeutics for retarding or preventing cataract, one of the most common (and costly) diseases of ageing.

Group Members

John McAvoy, BSc PhD, *Unit Co-Head*
Frank Lovicu, BSc PhD, *Unit Co-Head*
Richard Stump, BSc PhD, *Laboratory Manager*
Lavinia Taliana, BSc PhD, *Postdoctoral Fellow*
Jessica Boros, BSc, *Research Assistant*
Sharon Ang, BSc, *Research Assistant*
Michael O'Connor, BSc, *Postgraduate Student*
Elizabeth Wederell, BSc, *Postgraduate Student*
laxmi Iyengar, BSc, *Postgraduate Student*
Yongjuan Chen, BSc, *Postgraduate Student*
Colin Chong, MBBS, *Postgraduate Student*
Kevin Wang, BSc, *Postgraduate Student*
Bramilla Patkunathan, BSc, *Postgraduate Student*
Tracie Reinten, *Honours Student*

Research Activity

The lens transmits and focuses light onto the retina. To do this it needs to be transparent and to have appropriate refractive properties. This depends on the development and maintenance of highly ordered cellular architecture.

The lens consists of two forms of cells encapsulated within a basement membrane; (i) elongated fibre cells, grow to several millimetres in length,

and are precisely aligned to form a regularly packed spheroidal mass, and (ii) cuboidal epithelial cells form a single-layered sheet that covers the anterior surface of the fibres.

Whilst the fibre cells make up the bulk of the lens and mostly determine its optical properties, epithelial cells play a key role in maintaining an appropriate physiological environment within the lens. In addition, the epithelium contains the 'stem cells' that proliferate, migrate and differentiate into the new fibres that are progressively added to the fibre mass throughout life.

We have focussed our attention on growth factors because of their importance in regulating cell fates in developmental systems. Using a unique lens epithelial explant culture system we have identified members of the FGF growth factor family as inducers of lens cell proliferation, migration & differentiation; responses that are induced in a progressive dose-dependent manner. We have proposed that an anterior-posterior gradient of FGF determines lens polarity and growth patterns. A major thrust of research activity in our laboratory is aimed at testing this hypothesis.

Insights into the molecular basis of the major lens pathology, cataract, have also arisen from our growth factor studies. We have shown that members of the transforming growth factor beta (TGFß) family induce aberrant growth and differentiation of lens cells. This progressively leads to disruption of normal cellular architecture and opacification of the lens.

Cataract is the most common cause of blindness in the world today. Although surgery is generally effective, in many countries it cannot keep pace with the growing demand. Moreover, complications such as aberrant growth and differentiation of lens cells left behind after cataract surgery (most commonly referred to as posterior capsule opacification), require further treatment and add to the cost of cataract management. Because of its clinical significance it is vital to understand how TGFß is regulated in the eye and how it induces cataractous effects on the lens. This information is fundamental to understanding the molecular basis of cataract and devising strategies for prevention.

Projects in 2003

FGF signalling and lens cell proliferation and differentiation

Lovicu, Iyengar, Wang.

These studies were aimed at elucidating how FGF induces different cellular responses. We investigated the intracellular signalling cascades that are activated by FGF. We showed that the ERK1/2 (MAPK1/2) signalling cascade is involved in both proliferation and differentiation. Inhibition of ERK1/2 blocks cell proliferation; however, it blocks only the morphological (elongation), not the molecular (beta-and gamma-crystallin gene expression) aspects of differentiation. This indicates that fibre differentiation depends on activation of several intracellular signalling cascades. The dynamics of these growth factor-induced cascades were also studied in relation to the signaling cascades induced by the ocular media.

PDGF/IGF/EGF signalling and lens cell proliferation

Lovicu, Iyengar, Wang.

IGF/insulin and PDGF growth factor families can induce lens epithelial cells to undergo cell division. To determine if IGF and PDGF utilise the same signalling pathway(s) as FGF, we examined the role of ERK1/2 signalling in IGF- and PDGF-induced lens cell proliferation. Similar to FGF, both IGF and PDGF can induce lens cell proliferation, in an ERK-dependent fashion. However, PDGF activates ERK within 10 minutes compared to 20 minutes for IGF. Differences in the temporal activation of lens cell proliferation by IGF and PDGF may be due to differences in the mode by which these growth factors activate ERK1/2. The dynamics of these growth factor-induced cascades were studied in relation to the signaling cascades induced by the ocular media. Overall, these results support an important role for ERK signalling in growth factor-induced lens cell proliferation

Integrin expression patterns in lens development

Wederell, McAvoy, de Iongh (University of Melbourne).

In earlier studies we identified key cell-matrix

adhesion molecules in the lens. We were particularly interested in defining the changes in integrin gene expression during the transition of an epithelial cell into a fibre cell. The main findings were that alpha3 integrin, which is strongly expressed in epithelial cells, shuts off sharply as fibres begin to differentiate. In addition, alpha6A and alpha6B which are expressed in both epithelial cells and fibres, undergo a switch so that alpha6A is more strongly expressed in fibres than in epithelium. This indicates that different integrin family members may facilitate the different adhesion requirements of these two forms of lens cells to their substratum, the lens capsule.

BMP/Activin receptors and lens development
Chen, McAvoy, de Iongh (University of Melbourne).

The TGFß superfamily includes TGFß, BMPs and activins/inhibins. Our transgenic and explant studies have shown that signalling via TGFß receptors is important for lens fibre differentiation. BMPs have also been shown to play a role in lens induction. In this study we investigated the expression and signalling potential of BMP and activin receptors during lens development. The results suggest that during lens development there is signalling by several members of the TGFß superfamily. Up-regulation of the type I BMP receptor, Alk3, in fibres bereft of TGFß receptor signalling, is suggestive of a compensatory mechanism and further reinforces the notion that signalling by TGFß family members is required for terminal fibre differentiation.

Lens regeneration
O'Connor, Lovicu, McAvoy.

FGF is good at inducing fibre differentiation in lens epithelial explants. However, elongating cells in FGF-treated explants do not show the same degree of alignment with their neighbours as do elongating fibres in vivo. There is growing evidence to suggest that other, as yet unidentified factors, are involved in coordinating the differentiation of the epithelial 'stem cells' and their elongation into highly aligned fibre cells. Recent developments in tissue culture techniques pioneered by our laboratory are enabling a more rigorous investigation of the intraocular fluids in search of these factors. This raises the possibility of devising strategies that will promote lens reconstruction after cataract surgery.

Crim 1 expression and function in the lens
Lovicu, Boros, Little (University of Queensland), McAvoy.

We localised a new gene, Crim1 (initially identified by colleagues at University of Queensland), during eye morphogenesis. Crim1 is particularly strongly expressed in the lens epithelium. During postnatal growth, expression is reduced in all ocular tissues and eventually it is only expressed in the lens. Investigating the functional significance of Crim1 expression in the lens forms the basis of an ongoing collaborative study with colleagues at the University of Queensland.

TGFß-induced cataract & EMT
Lovicu, Steven (University of Kiel), McAvoy.

We used a transgenic model to study the effects of overexpressing TGFß in the lens. We showed that TGFß, in addition to alpha-smooth muscle actin & collagen types I & III, also induces desmin, fibronectin, & tenascin. These are all markers for subcapsular cataract and posterior capsule opacification in humans. In addition, normal phenotypic markers for lens epithelial cells are lost, including alpha-crystallin, Pax6 & connexin 43. The phenotypic changes induced by TGFß are more typical of connective tissue cells and indicate that TGFß induces an epithelial-mesenchymal transition (EMT). A similar EMT occurs in small eye mice (have a haploin sufficiency of Pax6) and indicates that TGFß induces EMT by down regulating Pax6.

Wnt signalling in lens development
Stump, Chen, Ang, Reinten, Lovicu, Pinson (University of California, Berkeley, USA), McAvoy.

We studied the expression of members of the Wnt growth factor family and its receptors, the frizzleds (Fzs). Wnts are commonly involved in regulating expression of molecules involved in cell proliferation, communication and adhesion. We showed that multiple Wnts and Fzs are expressed in the lens. In addition, we showed that key Wnt/Fz signalling molecules, including those involved in Wnt/ß-catenin and Planar Cell Polarity pathways, are expressed in the lens. A mouse mutant that is deficient in a co-factor required for Wnt signaling

does not develop a lens epithelium. This supported our hypothesis that Wnt signaling plays a key role in the formation and maintenance of the lens epithelial sheet. Promotion of Wnt signaling may provide an important means of protecting lens epithelial cells from the TGFß-induced epithelial mesenchymal transition that occurs in some forms of cataract.

Wnt signaling in TGFß-induced cataract
Chong, Ang, Lovicu, McAvoy.

Transforming growth factor-beta (TGFß) induces aberrant growth and differentiation in rodent lenses that is characteristic of some forms of human cataract. As changes in Wnt signalling are frequent key events in abnormal growth and dif ferentiation in other systems, we investigated Wnt expression in established TGFß-induced cataract models. W e showed that Wnt expression is altered in TGFß-induced rat and mouse cataract models. Levels of Wnt5a and Wnt7b protein and mRNA are distinctly upregulated in cataractous plaques of all the models we examined. This suggests that de-regulation of Wnt signalling is involved in regulating abnormal growth and dif ferentiation processes in TGFß-induced cataracts and may play a role in human cataract, such as posterior capsule opacification.

Vitronectin in lens development and cataract
Taliana, Ang, Evans (CSIRO, W est Ryde, NSW), McAvoy.

The lens capsule forms during lens morphogenesis and provides the substratum for lens cell attachment, growth and differentiation. Its primary extracellular matrix (ECM) constituents include type IV collagen, laminin, heparan sulphate proteoglycan, fibronectin and entactin/nidigen. Recent studies have shown that during the aberrant growth and differentiation seen in some forms of cataract, abnormal accumulations of ECM are deposited as lens cells undergo epithelial mesenchymal transition. One of the molecules identified within the resulting fibrotic plaques is vitronectin (Vn). We showed that Vn is expressed by lens epithelial cells and is present in lens capsule during development. Lens epithelial cells can attach and migrate on Vn; however , on this substratum, the cells undergo an epithelial-mes - enchymal transition that is characteristic of cataract. This result raises questions as to how, or if, lens cells and Vn interact in normal development and how this relationship may be disrupted during cataract formation.

Retinal Research Groups

The retina, which detects light like the film of a camera, is the most sophisticated and delicate part of the eye. Retinal diseases, such as diabetic retinopathy and age-related macular degeneration, are now the commonest causes of untreatable blindness in Australia.

The SSI has 3 research groups working on various aspects of the retinal biology and pathology –

1) Retinal Development, Aging & Eye Cancer
2) Retinal Therapeutics
3) Retinal Cell Death and Survival

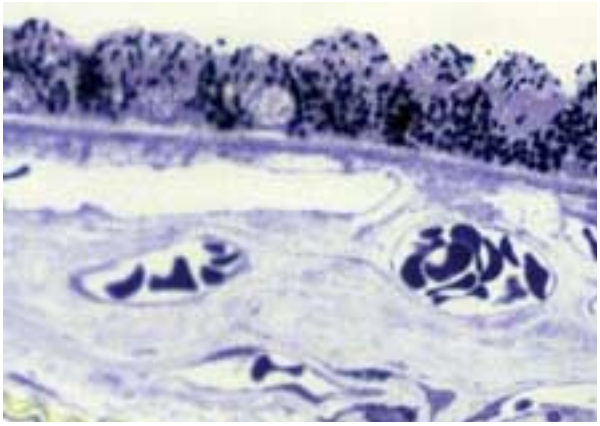
The groups work in close association – sharing information, insights and frequently joint publications.

Age-related Macular Degeneration is a common thread of research in terms of:

•Basic biology, development and changes in associated vasculature.

•The importance of therapy for sub-retinal new vessels in the blinding stage of this disease.

•The fact that genetic factors may be important in susceptibility to the disease.



Light micrograph of normal retina

Retinal Development, Aging & Eye Cancer Research Group

Research aims to shed light in 2 areas - Retinal Development and Eye Cancers - and to explore the inter-relationships between them.

Group Members

Jan Provis, BSc PhD, *Unit Co-Head*
Michele Madigan, B.Optom PhD, *Unit Co-Head*
Diana van Driel, BSc, *Senior Research Assist.*
Alexandra Allende MBBS, *Postraduate Student*
Pierre Georges, BSc, *Postgraduate Student*
Trong Van Pham, MD, *Postgraduate Student*
Trent Sandercoe, BSc, *Postgraduate Student*
Edward Chao, BSc, *Honours in Medicine candidate*
Kenneth Lai, BMedSci, *Hons Postgraduate Student*

Members advising on clinical aspects:
Max Conway, PhD, FRACO, FRACS
Meidong Zhu, PhD, *Research Fellow*

Research Activity

The human 'macula' is a region of the retina that includes several specialisations not present in non-primate retina and which together enable us to see detail. Understanding the development and aging of the macula and how this unique structure makes humans vulnerable to degenerative disease is the principle aim of our research into development and aging. The fovea is at the geometric centre of the macula and is identifiable by a number of structural modifications including: (1) an absence of a direct blood supply from the retinal circulation, (2) the presence of a pit which thins the retina locally, (3) a focal concentration of a class of colour sensitive photoreceptors (cones), and (4) the inclusion of specialised circuitry which conserves the electrical responses of individual cones. Few groups internationally are applying modern molecular tools to the investigation of primate retinal development and human retinal disease. During 2001 we have developed new approaches to investigate the biology of the macula. Our focus is to identify unique molecular profiles associated with the unique anatomy and physiology of the macula and fovea.

Projects in 2003

Genechip™ Analysis of Genes Regulated by Hyperoxia in the Mouse
Natoli, Provis, Stone.

Oxygen is an important component in both the development and maintenance of the eye. The regulation of oxygen in the eye is important because an increases in normal oxygen tension (hyperoxia) or decrease (hypoxia) can cause death of retinal photoreceptors. Such mechanisms may have a role in diseases such as retinal dystrophy and macular degeneration. We are studying the genes regulated by hyperoxia in C57/Bl mice, using gene microarray technology. The results indicate that expression of a family of heat shock proteins is dramatically decreased in hyperoxia, suggesting that these genes may have a role in regulation of oxygen in the retina.

Growth Factors in Retinal Development
Allende, Chao, Natoli, Madigan and Provis.

Fibroblast growth factors regulate a number of cell functions including morphological differentiation (ie changes in cell shape). At the fovea – the part of the retina that enables us to discriminate fine detail – cones are highly elongated and very narrow, enabling them to pack at high density. During aging and in macular degeneration this morphological specialisation is lost and is associated with a decline in visual acuity. Our studies show that during development members of the Fibroblast Growth Factor (FGF) family are expressed on cone photoreceptors in a pattern consistent with a role in cone differentiation. In particular, cone photoreceptors uniquely express one of the receptors with high specificity for only one member of the FGF family. Other studies show certain members of the Transforming Growth Factor (TGF) family are expressed in the developing retina in a gradient that suggests they may have a role in definition of the macula.

Blood Vessel Growth During Foveal Development
Allende, Madigan, Provis.

Other studies show that certain members of the Transforming Growth Factor (TGF) family are expressed in the developing retina in a gradient

that suggests they may have a role in definition of the macula. W e are testing the hypothesis that TGFß is highly expressed in this region and inhibits angiogenesis .

Eye Cancers: Retinoblastoma (Rb) and Ocular Melanoma are the most common primary intraocular eye cancers in children and adults respectively. Better understanding of the pathogenesis of these tumours involves understanding the normal processes of development and aging in the retina and choroid, as well as the mechanisms controlling proliferation, cell death and angiogenesis within tumours. Combined clinical and laboratory research provides an understanding of the biology of these tumours and improves the rationale for treatment. This is important given the morbidity associated with enucleation, the side ef fects of therapies, particularly radiation, and the high incidence of metastases in ocular melanoma

In vivo effects of retinoids on tumour growth in an Rb mouse model
Madigan, Conway , van Quill (Ocular Oncology , UCSF, San Francisco), O'Brien (Ocular Oncology, UCSF, San Francisco).

Collaborative studies with Professor O'Brien are investigating the ef ficacy of various retinoids in controlling intraocular tumour growth in a mouse model of Rb. These agents can induce tumour cell differentiation and inhibit tumour growth in some instances. When used in combination with chemotherapy and radiation, some of these agents may reduce the dose of potentially cytotoxic therapies required when treating tumours.

Targeted immunoradiotherapy of ocular melanoma
Madigan, Conway , Billson, Li, Allen (St George Hospital, University of NSW).

Laboratory research has focused on using select antibodies or proteins to target radiotherapy more precisely to melanoma cells to deal with both primary and metastatic disease. W e have recently been studying a melanoma specific antigen NG2 (using Mab 9.2.27) which may have potential as a target for radiotherapy treatment. Preliminary studies show that the majority of choroidal melanomas and ocular melanoma cell lines (from Prof Jager, Netherlands) express NG2 immunoreactivity. In vitro studies indicate specific cytotoxicity of alpha-immunoconjugates (213Bi-9.2.27) for NG2-positive melanoma cell lines, suggesting potential use in local therapy either alone or in combination with plaque radiation.

Matrix metalloproteinases in ocular melanoma
Madigan, Lai, Crouch, Conway , Jager (Leiden Medical Centre).

Matrix metalloproteinases (MMPs) are a family of enzymes involved in degradation of extracellular matrix components such as collagen, laminin and proteoglycans. MMPs are involved in normal development, wound healing, new blood vessel growth and tumour invasion and metastases. Our studies in ocular melanoma indicate that MMP-2, -9 and MT1-MMP are involved in tumour angiogenesis. W e have also found that ocular melanoma cells express EMMPRIN, an inducer of MMP activity. The juxtaposition of EMMPRIN-positive tumour cells and MMP-2 expressing fibroblasts at tumour edges provides insight into the mechanisms underlying ocular melanoma growth. In vitro studies support these findings, where co-cultures of fibroblasts and melanoma cells display enhanced MMP-2 activity compared to fibroblasts or melanoma cells grown alone.

The Effect of UV on Growth of Uveal Melanoma
Lai, Di Girolamo (UNSW) and Madigan.

Epidemiological studies suggest that exposure to UV radiation has a role in the pathogenesis of uveal melanoma. Preliminary studies have investigated the ef fects of UV -B radiation on ocular melanoma cell growth, viability and MMP-production. Melanoma cells appear to be very sensitive to UV-B radiation and cell death is induced even at very low doses (~5mJ/cm2). However , lower doses of UV-B do not appear to induce significant effects on MMP production by ocular melanoma cells. Whether UV -B can induce changes in primary choroidal melanocytes important for transformation to melanomas, as seen for example in cutaneous melanoma, is currently being investigated.

Development of an artificial eye
Conway, Ben-Nissan (University of Technology).

The consequences of some ocular diseases, and especially tumours, may be the loss of an eye with significant functional and psychological sequelae. Hydroxyapatite is currently used to replace eyes lost due to any cause, however it may be associated with problems including the potential for extrusion, infection, reduced motility and loss of cosmesis over time. The group has developed a novel porous bioceramic with improved biointegration and mechanical properties, which promises better function and cosmesis over existing implants . The results of the physical studies have recently been published; in vivo evaluation is in progress.

Retinal Therapeutics Research Group

This group aims to understand how the permeability of the retinal blood vessels is controlled in order to identify new and improved treatments for swelling of the retina, which is a common cause of loss of vision in conditions such as diabetic retinopathy. The Retinal Therapeutics Research Group includes both clinical and laboratory research units.

Group Members

Mark Gillies, MB BS PhD FRACO, *Director of the Unit*

Clinical:
Maria Males, RN BN BA G.dip (Acute Care Nurs) *Clinical Research Officer*
Haipha Ali, BSc (Applied V ision Sciences, Orthoptics)
Meidong Zhu, MD PhD, *Senior Research Fellow*

Laboratory Research:
Martin Windsor, PhD, *Senior Research Fellow*
Li Wen, MB BS M Med, *Research Officer*
Bryony Stracey, *Technical Officer*
Svetlana Cherepanof f, BMed Sci MB BS, *Postgraduate Student*
Goff Quin, MB BS, *Postgraduate Student*
Marina Tretiach, BAppSc, *Postgraduate Student*
Jenny W yndham, BSc DipEd MS MPH, *Postgraduate Student*

Research Activity

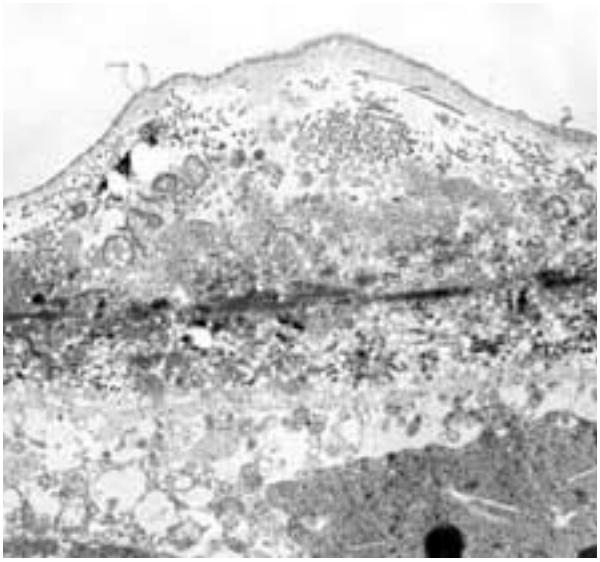
Clinical Trial Group

The Retinal Therapeutics Research Unit is an international certified clinical trial centre that conducts randomised clinical trials and other clinical and basic research in retinal disease. Currently , four pharmaceutical company sponsored and three investigator initiated clinical trails are undertaken in the Unit. These study trials involve the researches in the treatment and mechanisms of age-related macular degeneration and diabetic retinopathy.

Laboratory Research Group

Retinal vascular diseases caused by abnormal leakage of fluid through small blood vessel walls (capillaries) are among the commonest cause of blindness – especially in diabetic retinopathy, retinal vein occlusion, ocular inflammation and the wet form of AMD.

The retinal therapeutics group has developed several assays for studying the bimolecular determinants of leakiness in retinal capillaries. The group also conducts pre-clinical studies into pharmacological and other therapies for the treatment of retinal vascular disease.



Electron micrograph of a druse in the retina

Projects in 2003

A randomised clinical trial of intravitreal triamcinolone for refractory diabetic macular oedema

Gillies, Zhu, Ali, Males, Sirimaharaj*, Sutter#
*Dr Maytinee Sirimaharaj is a V isiting Retinal Fellow in SSI 2003/04
#Dr Florian Sutter was a Vsiting Fellow in 2002/03 in SSI

Macular oedema is the leading cause of vision impairment from diabetic retinopathy. Laser treatment has been proven ef fective in reducing the risk of visual loss from diabetic macular oedema and is widely employed, but suffers the deficiency that it is inherently destructive. Progressive loss of vision occurs in up to 26% of patients with diabetic macular oedema despite laser treatment. There have been some uncontrolled and anecdotal reports recently that an intravitreal injection of triamcinolone (IVTA) may result in reduction of diabetic macular oedema and improved visual acuity. This project is a prospective, double-masked, placebo-controlled randomised clinical trial to conduct a 2-year, single-centre, clinical trial of the efficacy and safety of intravitreal injections of triamcinolone acetonide for diabetic macular oedema that has failed laser treatment. Sixty-nine eyes of 43 patients have entered into the study . The results of this study will be directly relevant to patients with type I diabetes since it will provide data for use by clinicians that may reduce the risk of blindness from diabetic retinopathy.

Chorioretinal venous anastomosis for non-ischaemic central retinal vein occlusion

McAllister[1], Gillies[2], Mitchell[3], Zhu[2], Ali[2], Males[2]
1.Lions Eye Institute, Western Australia
2.Save Sight Institute/Sydney Eye Hospital
3.Westmead Hospital, Sydney

Central retinal vein occlusion (CR VO) is a frequent cause of visual loss, especially in the elderly. Obstruction of central retinal vein may be partial or complete and can produce non-ischemic or ischemic CRVOs. The non-ischemic form initially is more common and vision is reduced due to retinal oedema. Even where the obstruction is eventually overcome, the prognosis for full return of central vision is poor due to macular damage from chronic oedema. A technique of venous anastomosis between the retina and choroid, using high intensity laser as a means of bypassing the obstruction has been created and that has been able to improve the success rate to 60% in a small trial over 5 years. The current project is a 3 year prospective, multicenter , randomised clinical to examine whether the use of high powered laser to create retinal vein bypass will benefit patients with a blockage of the central retinal vein. SSI is one of the main centre for the trial. W e have enrolled 23 patients and 20 patients have reached their endpoints. The data analysis is ongoing.

Intravitreal Triamcinolone versus Laser for Macular Oedema Secondary to Retinal Vein Occlusion

Gillies[1], Larson [1,2]*, Zhu [1], Hvarfner [1], Sutter [3], Ali [1], Males[1]
*A/Prof Jorgen Larson is a visiting fellow currently working in the SSI
1.Save Sight Institute
2.Department of Ophthalmology , Lund University Hospital, Lund, Sweden
3.Department of Ophthalmology, Zürich University Hospital, Switzerland

An occlusion of a vessel in the retina (retinal vein occlusion) leads to damage of blood vessels, causing them to leak resulting in oedema of the macula. The macular oedema is the main cause of loss of vision in eyes with retinal vein occlusion. Laser treatment can in some patients improve the visual acuity, but does not work in all cases. W e have conducted a randomised clinical trial which has demonstrated that, at least in the short term, injection of a steroid, triamcinolone, into the eye reduces macular oedema and improves vision in 2/3 patients with macular oedema caused by diabetes that had persisted despite laser treatment. In many cases the oedema completely disappeared. This project is to conduct a 2 year , prospective, multi-centre, double-masked, exploratory, randomised, placebo-controlled trial in patients with branch retinal vein occlusion in order to assess if intravitreal triamcinolone is superior to laser treatment. V ariables related to the progression or resolution of macular oedema will be examined in the study eye of all patients to determine the ef fects of treatment. In the meantime, we are recruiting patients.

A phase IIIb randomised, double-masked, active controlled, dose-ranging, multi-centre comparative trial, in parallel groups, to compare the safety and efficacy of intravitreal injections of eye001 (pegaptanib sodium, macugen) given every 6 weeks for 102 weeks, to pdt with visudyne, in patients with exudative age-related macular degeneration (AMD)
Sponsored by Eyetech Pharmaceuticals
Gillies, Males, Zhu, Luo, Ali, Sirimaharaj*
* Dr Maytinee Sirimaharaj is a V isiting Retinal Fellow in SSI 2003

This is a multicentre, international, placebo controlled trial studying the safety & ef ficacy of an Anti-Vascular Endothelin Growth Factor Aptamer injected into the eye in patients with wet Age Related Macular Degeneration. In 2003, 16 SEH patients underwent their 2nd year of the study.

A phase II randomised, controlled, double-masked, dose-finding, multi-centre, comparative trial, in parallel groups, to establish the safety and preliminary efficacy of intravitreal injections of EYE001 (Anti-VEGF Pegylated Aptamer), given every 6 weeks for 12 to 30 weeks to patients with clinically significant diabetic macular edema (CSME) involving the centre of the macula. (HREC Ref 02/311)
Sponsored by Eyetech Pharmaceuticals
Gillies, Males, Zhu, Ali

This is a multicentre, international, placebo controlled trial studying the safety & ef ficacy of an Anti-Vascular Endothelin Growth Factor Aptamer injected into the eye in patients with diabetic macular oedema. 3 SEH patients were enrolled and completed their treatment phase. Post treatment monitoring continues in this ongoing trial.

The effect of paravascular cells on retinal permeability
Tretiach, Gillies.

It is likely that cells situated near the retinal blood vessels can influence permeability. In this project, Müller cells and pericytes have been co-cultured in our laboratory model of retinal vascular permeability. In addition to assessing the effects of these cells on vascular permeability, the ef fects of various conditions that mimic human disease such as low oxygen levels have also been assessed.

The effect of retinal laser treatment and pharmacological intervention on the permeability of retinal vessels
Quin, Windsor, Tretiach, Gillies.

While laser treatment of retinal swelling is often a very effective treatment, its mode of action is not at all understood. We propose that laser treatment induces the secretion of a protein that stabilises the “blood-retinal barrier”. Preliminary laboratory experiments in which various retinal cell types are added to the vascular permeability assay suggest that this may indeed be the case.An animal model of early leak in the diabetic rat retina has been characterised to explore the effects of retinal laser treatment more fully. The identification of a barrier-restoring factor may have a therapeutic potential.

The role of oxidative damage in diabetic retinopathy
Van Reyk, Gillies.

Whilst it is suspected that oxidative stress may contribute to diabetic retinopathy, the identification of those antioxidants that could be developed as a treatment is hampered by the lack of understanding of which of the many potential oxidative pathways in the retina is involved, and precisely what is being damaged. W e are using high performance liquid chromatography to detect the target types of oxidative damage to retinal proteins. This will identify the critical pathways involved which will then be targeted by specific antioxidants in animal studies.

Autoantibodies in macular degeneration
Cherepanoff, Gillies.

Antibodies directed against retina are known to occur in the age related macular degeneration. This project will determine whether these antibodies have any effect on clinical outcome. A range of patient sera is in the process of being screened and the results will be correlated with the visual outcomes.

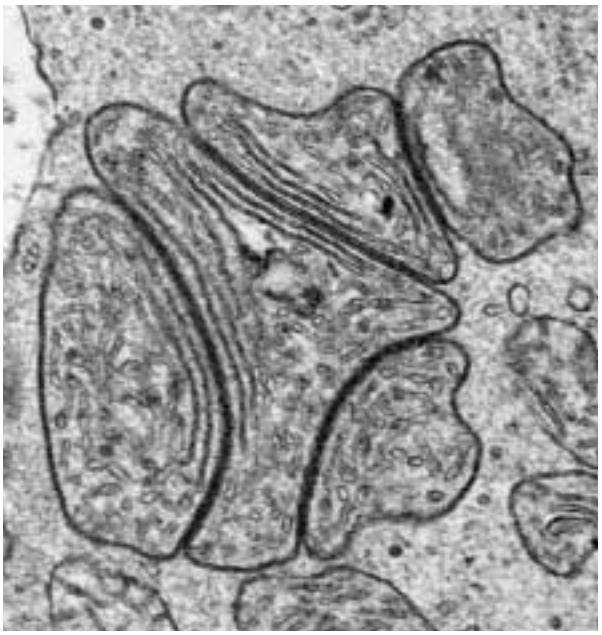
The role of ‘matrix metalloproteinase (MMPs) in the control of retinal vascular permeability
Wyndham, Gillies.

MMPs are a group of enzymes that can digest extracellular materials as well as cell surface

receptors. The presence of MMPs was characterised in retinal vascular cells. The results indicate that MMP activity may be involved in the development of retinal swelling in diabetes, and that matrix metalloproteinase inhibitors may represent a new treatment for diabetic retinopathy.

Connective tissue growth factor and retinal vascular disease
Wen, Gillies.

CTGF is a recently characterised growth factor which has an activity consistent with the regulation of blood vessel growth. We have found that retinal vascular cells become leakier when treated with CTGF. We have also found that CTGF increases the sensitivity of choroidal vascular cells to the leak-inducing factor VEGF . These findings suggest that CTGF may have a role in the development of vascular eye disease.



Electron micrograph of photoreceptor synapses

Retinal Cell Death and Survival Research Group

This group, now located in the Research School of Biological Sciences at the Australian National University, has been associated with the Save Sight Institute since 2002. The group has several collaborative projects with Save Sight Institute researchers and a growing track record of joint publications.

Group Leaders

Jonathan Stone, BSc PhD FAA, *Unit Head*
Krizstina Valter, MD PhD, *Senior Research Officer*

Research Activity

Research concerns the mechanisms which control the death and survival of neurones in degenerative retinal disease.

The group has made a series of findings novel in the understanding of photoreceptor death and survival in a range of rodent models and in the human.

New techniques and collaborations have been developed to take the analysis of the mechanisms of photoreceptor disease to the molecular level and to include the epidemiology of human disease.

Clinical trials have been organised to deploy some of this knowledge.

Projects in 2003

Current joint projects include the examination of mitochondrial specialisations in the normal and degenerative retina, and clinical trials of light restriction.