Save Sight Institute Research Reports 2002

Introduction:

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

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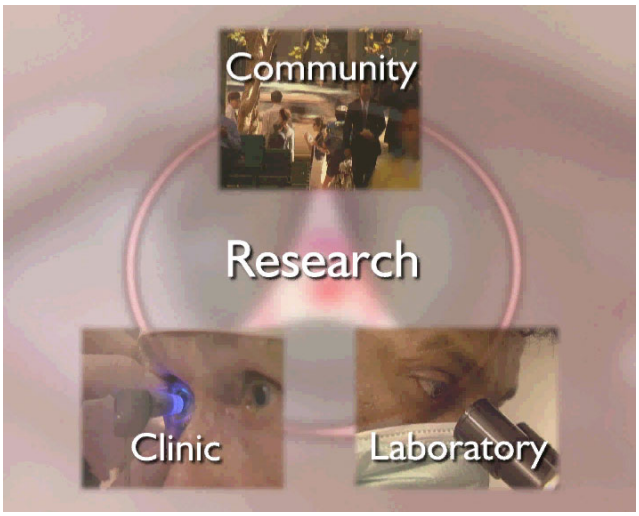

Community, Clinic and Laboratory Research – a powerful interface

Clinical Epidemiology Research Group

This group's research focuses on the causes, impacts, frequency and risk factors for common age-related eye diseases such as AMD, cataract, glaucoma, diabetic eye disease and other vascular problems affecting the eye. Visual impairment caused by these diseases, together with under-corrected refractive error, exerts a substantial impact on the independence, aged care service utilization and quality of life of older Australians. It also contributes to premature nursing home admission and mortality. Because of increasing life expectancy, age-related sensory loss now represents one of our most important medical challenges. Generation of accurate data on the prevalence, incidence and risk factors is a critical step in responding to this challenge.

Group Members (Centre for Vision Research, Westmead campus)

Paul Mitchell, MD BS PhD FRACO FRACS FRCOphth FAFPHM, *Unit Head*
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Research Activity

The Blue Mountains Eye Study (BMES) was the first large population-based assessment of visual impairment and common eye diseases in a representative older Australian community. This project has now provided reliable Australian estimates of the frequency (prevalence) of eye disease, refractive error and visual impairment, and has assessed their principal risk factors.

The study also provided the first large-scale Australian data on the frequency and risk factors for age-related hearing loss through a

separate project, the Blue Mountains Hearing Study, conducted in collaboration with members of the Department of Linguistics at Macquarie University. We also collaborated with researchers from the University of Wisconsin-Madison, USA, to align key measures of eye disease and hearing loss with a similar US study, conducted in Beaver Dam, Wisconsin.

BMES has extensively studied the diet of this older population to assess nutrition and diet-eye disease links. Longitudinal assessments of fractures, vascular events and mortality in this community, including links with sensory loss, are also underway.

Projects in 2002

Blue Mountains Study:

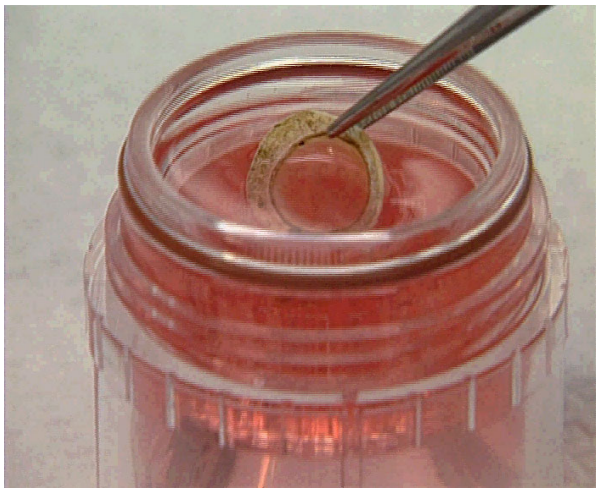
- Completing a number of phases of the main study.
- Reporting data from the 5-year examinations of participants initially seen during 1992-4 & reviewed during 1997-9.
- Reporting data from the Hearing Study.
- Completing an extension project involving assessing new residents to the area and those who had recently turned 50.

Cataract & Memory Study: Designed a NHMRC-funded trial aimed at determining whether there are cognitive benefits from cataract surgery. It is being conducted in association with Prince of Wales Medical Research Institute and is recruiting patients from both Prince of Wales and Westmead Hospitals.

Retinal Vein Bypass Study: This NHMRC-funded collaborative trial across 3 sites (Perth, Sydney & Melbourne) aims to establish whether a laser-induced artery-vein bypass will benefit people who develop central retinal vein occlusion, a largely untreatable cause of vision loss in the older age group.

Cornea Research Group

Corneal research in the Institute is directed towards investigating the pathogenesis of two important causes of corneal blindness, infectious keratitis and keratoconus.



Corneas are stored in specially prepared media

Group Members

Kathy McClellan, MBBS PhD FRACO FRACS, *Unit Head*
Con Petsoglou, *MBBS, FRACO, FRACS, Lecturer*
Raj Devasahayam, BAppSc, *Eye Bank Laboratory Manager*

Research Activity

The cornea is that part of the anterior eye wall, modified for light transmission via the lens to the retina. The cornea contributes significant refractive power to the eye and must remain transparent and regularly shaped to fulfill these functions.

The corneal surface is covered by stratified non-keratinising epithelium, which is uniquely placed in an external mucosal environment. This location must be maintained by an adequate circulation of tears from the lacrimal gland as well as by a healthy conjunctiva and eyelids that can close completely and facilitate tear drainage via the lacrimal canalicular system. In addition the cornea must have an intact sensory nerve supply to activate these mucosal defences and maintain an intact surface epithelium.

We have shown that humans can initiate an immune response to cysts of *Acanthamoeba* sp, the organism responsible for an intractable keratitis in contact lens wearers and determined enzymes important in the development of keratoconus. These studies will increase our understanding of these disease

mechanisms and direct strategies for prevention.

Projects in 2002

Immune Responses to Acanthamoeba Cysts: *McClellan*. Corneal infection can result from a failure of surface defences and may be caused by bacteria, viruses, fungi or amoebae. The scarring that results from corneal inflammation remains an important cause of world blindness.

Acanthamoeba remain an important cause of corneal infection amongst contact lens wearers in developed countries. The infection persists in the cornea causing severe pain and significant inflammation that frequently causes blindness due to irreversible corneal scarring. The organism exists in two forms, trophozoites and cysts and residual *Acanthamoeba* persisting in the cornea may reactivate after corneal transplantation.

The persistence of *Acanthamoeba* in the cornea indicates that the organism escapes immunological elimination and the aim was therefore to determine the immunogenicity and antigenicity of the *Acanthamoeba* cyst. Systemic humoral and cell-mediated immune responses were measured in C57BL/6 mice after intraperitoneal immunization with *Acanthamoeba castellanii*. Serum anti-*Acanthamoeba* IgG was measured by ELISA.

Lymphoproliferative assay and delayed type hypersensitivity responses to *A. castellanii* cyst and trophozoite antigen were used to determine the cell mediated immune responses generated by the *Acanthamoeba* cyst. *A. castellanii* cysts were both immunogenic and antigenic in this mouse model, producing anti-*Acanthamoeba* serum IgG, T lymphocyte proliferation and delayed type hypersensitivity responses. These results indicate that *Acanthamoeba* cysts are recognized by the immune system. The persistence of the organism in the human cornea means that these adaptive immune responses fail to kill *Acanthamoeba* cysts.

This is the first demonstration that *Acanthamoeba* cysts are both immunogenic and antigenic. The information enables a better understanding of the chronicity of the human infection. It suggests that the most rapid elimination of stromal cysts together with therapies to control corneal inflammation, will most effectively limit the destructive effects of *Acanthamoeba* keratitis.

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 FACS, *Unit Head*
Nitin Verma, MD (Oph) FRACO
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Max Conway, PhD FRACO FRACS

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. A digitally equipped mobile screening unit is being trialed in NT as a model for a similar initiative in Broken Hill and remote regions of Australia.
- ***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 2002

Tele-ophthalmic Diabetic Eye Screening Program:

Aboriginal Health worker Dot Butler using digital media to screen for Diabetic Retinopathy in NT.

In 2002, postgraduate student I-van Ho completed the implementation and cost-effective analysis of the Tele-ophthalmic Diabetic Eye Screening Program in the remote Aboriginal communities of the Top End of Northern Territory.

This program centred around a Mobile Diabetic Eye Screening Unit comprised of an opthalmically trained Aboriginal Health Worker and Registered Nurse and equipped with a Digital Non-Mydriatic Fundus Camera with archiving and Tele-ophthalmic capabilities. The capturing and transmitting capability of the unit allowed all retinal images to be electronically sent to Darwin for assessment of the need for specialist treatment as well as confirmation of diagnosis by ophthalmologists based there.

The team conducted a detailed accuracy analysis which demonstrated that the Digital Fundus Imaging system is comparable to conventional 35mm Slide Fundus Photography for screening and grading diabetic retinopathy. The analysis confirmed that the digital technology is highly sensitive and specific in detecting sight threatening retinopathies.

The Mobile Tele-Ophthalmic Team covered over 20,000km to provide diabetic eye screening services in 19 out of 32 remote Top End communities. It reviewed 370 out of 584 diabetic patients in remote communities and significantly improved the diabetic eye screening coverage rates from 44% to 76% across the population, with several communities achieving 100% screening coverage rate.

The Mobile Diabetic Eye Screening Team have developed the most updated and extensive database on all the diabetics in the Rural Aboriginal communities of the Top End. They are now expanding and providing a more comprehensive and *holistic* service to the communities by integrating the database with the Territory Health Service Community Diabetic Register.

The mobile eye team now works in close partnership with Nutritionists, Diabetic Educators, Endocrinologists, General Surgeons and Renal Physicians, in the form of Specialist Outreach Services, to provide comprehensive diabetic care for the patients and establish an efficient screening and primary referral unit for specialist diabetic treatment.

From The data collected to date, Tele-Ophthalmology holds promise to be the future in delivery of Ophthalmic Services to rural and remote Australia. With completion of the collection of data from all 32 communities in the study in the coming year it is hoped not only to further confirm this, but also to demonstrate the cost effectiveness of the program.

Virtual Ophthalmology Clinic: 2002 saw the completion of education trials using the unit's virtual ophthalmology program which allows medical students to gain skills in history taking, making use of "virtual" clinical patients. This computer-based program mimics real interactions with patients, in that students have complete control of the order in which they ask questions and gather information, to make a diagnosis before an examination. The information can then be emailed to the supervisors and mentors of the program and when a provisional diagnosis is made, the student is then allowed to continue their examination and suggest investigations. The program has the potential to be delivered via the Internet and further work has been done towards achieving this. The strength of the program is that it allows the candidate to develop skills in interviewing and forming a diagnosis, sparing both the patient and the student the stress of learning these skills in a live situation. This is seen as an adjunct or prelude to patient contact for the students.

Electrophysiology & Glaucoma Research Group

Glaucoma causes painless irreversible peripheral vision loss and is a major cause of blindness in developed countries. This group has developed a technique that assesses peripheral vision objectively and has the potential to detect glaucoma at an earlier stage than previously possible. Further research will investigate this technique in the detection and ongoing monitoring of glaucoma.

Group Members

Alexander Klistorner, BMed PhD, *Unit Co-Head*
Stuart Graham, FRACO, *Unit Co-Head*
Frank Billson, FRACO, FRACS
John Grigg, FRACO, FRACS
Lisa Feldman, Dip Nursing, *Electrophysiology Technician*
Chandra Balanchandran, MBBS, *Postgraduate Student*
Allesandra Martins, MBBS, *Postgraduate Student*
Alejandro Estrada, MBBS, Visiting Fellow

Objective mapping of the visual field using technology developed by SSI.

Research Activity

The electrophysiology group has been conducting research into new methods for detecting glaucoma and other causes of visual loss. A new technique to objectively detect visual field defects in glaucoma has been developed and is known as the multifocal pattern visual evoked potential (MPVEP). This involves the use of an electrode array oriented around the occipital region of the skull recording multiple channels of data from the visual cortex, and utilises a new stimulating and recording algorithm, to test all parts of the visual field simultaneously.

The MPVEP is able to objectively identify the extent of glaucomatous damage and may be able to detect changes before subjective field loss occurs. Clinical trisals were conducted in glaucoma and high risk suspects comparing conventional techniques with the MPVEP, FDT perimetry, SWAP perimetry and Scanning Laser Polarimetry of the nerve fibre layer. Information from these trials will help determine which of these methods, or combination thereof, is superior for early detection of glaucoma.

Refinement of the methods for the early detection of glaucoma using the multifocal pattern VEP (AccuMap): *Klistorner, Graham, Martins*. Together with established black/white pattern stimulation, blue-on-yellow and low contrast stimulations were used in order to investigate specific pathways (Magnocelluar and Blue-Yellow), which are affected very early in a disease. Other markers of the early disease such as the subjective functional investigations of short-wave-length perimetry, and frequency doubled perimetry were compared to the objective anatomical evaluation of the optic nerve using Heidelberg Retinal Tomography. These tests were used to compare with multifocal VEP results. A database of normal subjects, including mean and SD values of VEP amplitude for every stimulated area of the visual field, were available for comparison. Points with a difference in amplitude of more than 1.96 standard deviations (ie $p<0.05$) from the mean value for that point in the normal database were judged abnormal. The use of the technique in children was another area, which was assessed - children as young as 5 years of age can reliably perform the test. Different modifications of the software were tested in order to expand its use to even younger individuals.

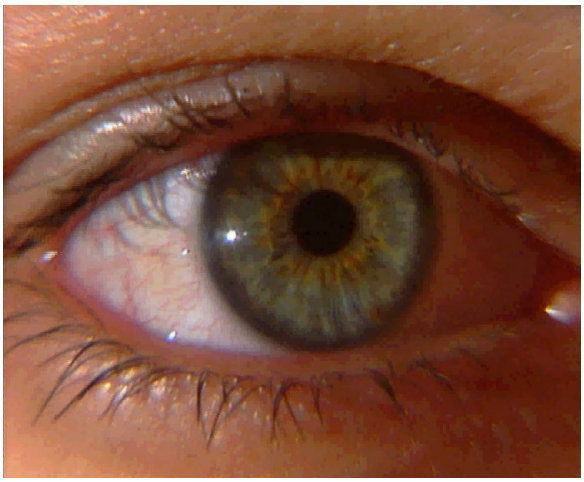
Development of new techniques for detecting visual loss and its use in detecting optic neuritis (multiple sclerosis) and optic gliomas in children: *Klistorner, Graham, Balachandran*. Preliminary research conducted at SSI on subjects with clinically definite MS involving the optic nerve showed very promising results for detecting and monitoring this disease. These studies were extended as follows. First the current database of 100 normals was assessed to derive a new algorithm for latency analysis considering the various waveforms collected by the multichannel input. VEP testing of subjects with clinically definite MS involving the optic nerve (onset>6 months ago) were conducted to establish the typical range of amplitude defects and latency delays seen in chronic disease. Results were correlated with MRI scan data where available. This will be followed by studies

on acute optic neuritis subjects (first episode), to be recruited from the SSI clinic and referring neurologists and ophthalmologists. There are also plans to investigate the potential of the technique in monitoring brain tumours.

In 2002, good progress was maintained by ObjectiVision LTD (University and SSI are the major shareholders) to commercialise development of the method of objective detection of the visual field defects. Alex Klistorner & Stuart Graham developed & patented a scaling algorithm to reduce inter-subject variability of VEP amplitude.

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*
Oonagh Lynch, BSc PhD, *Postdoctoral Fellow*
Heidi Brown, 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*
Nagalaxmi Iyengar, BSc, *Postgraduate Student*
Yongjuan Chen, BSc, *Postgraduate 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. Our studies 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, 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 2002

FGF signalling: *Lovicu,Iyengar.* 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.

PDGF/IGF signalling: *Lovicu, Lynch, Iyengar.* 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. Overall, our results support an important role for ERK signalling in growth factor-induced lens cell proliferation.

Integrin expression patterns: *Wederell, McAvoy, de Iongh (University of Melbourne).* In other 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: *Brown, de Iongh (University of Melbourne), Chen.* The TGFß superfamily includes TGFß, BMPs and activins/inhibins. Our 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 fiber 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: *Lovicu, 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, 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

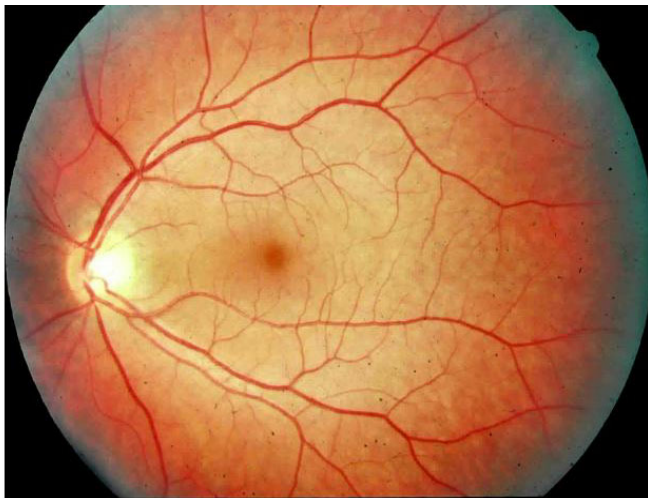
alpha-smooth muscle actin & collagen types I & III, also induces desmin, fibronectin, & tenascin. These are all markers for subcapsular cataract and posterior capsule opacification in humans. In addition, normal phenotypic markers for lens epithelial cells are lost, including alpha-crystallin, Pax6 & 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 haploinsufficiency of Pax6) & indicates that TGFß induces EMT by down regulating Pax6.

Cell proliferation in TGFß-induced cataract: *Ang, Lovicu.* The role of cell proliferation in the growth of subcapsular plaques in our cataract model (transgenic mice overexpressing TGFß) was investigated. BrdU incorporation studies revealed that plaques grow by proliferation and migration of cells at their periphery. After a short incubation time with BrdU, cells within plaques were rarely labelled, indicating that cell proliferation ceases once cells become incorporated within them. Cells at the plaque periphery express the cell cycle inhibitor Kip2, just prior to their differentiation within the plaque. This study shows that the growth of cataractous plaques depends on recruitment of cells from the apparently normal regions of the epithelium.

Wnt signalling: *Stump, Ang, 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 have shown that multiple Wnts and Frzs 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 supports 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.

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 &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.

Retinal Development, Aging & Eye Cancer Research Group

Research aims to shed light in 3 individual areas and explore the connections between them –

- Retinal Development
- Age-related Macular Degeneration
- Eye Cancers

Group Members

Jan Provis, BSc PhD, *Unit Co-Head*
Philip Penfold, BSc PhD, *Unit Co-Head*
Michele Madigan, B.Optom PhD, *Unit Co-Head*
Diana van Driel, BSc, *Senior Research Assist.*
Li Wen, Bmed, MMed, *Research Assistant*
Alexandra Allende MBBS, *Postraduate Student*
Elisa Cornish, BSc, *Postgraduate Student*
Pierre Georges, BSc, *Postgraduate Student*
Van Pham, MD, *Postgraduate Student*
Trent Sandercoe, BSc, *Postgraduate Student*
Kenneth Lai BSc Hons candidate
James Walcott BSc Hons candidate
Edward Chao BSc Hons in Medicine candidate

Members advising on clinical aspects

Frank Billson, FRACO, FRACS, FACS
Max Conway, PhD, FRACO, FRACS
Matthew Healey, BSc, *Honours Student*
Andrew Chang, FRACO
Meidong Zhu, PhD, *Research Fellow*

Research Activity

Retinal Development:

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.

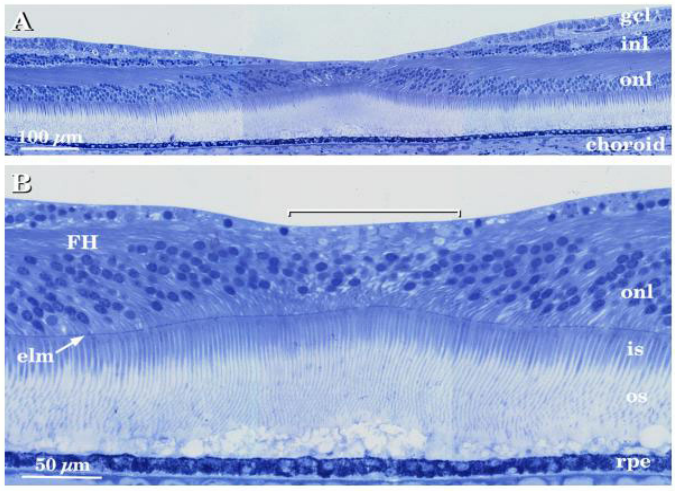
The fovea is at the geometric enter of tl macula and is identifiable by a number o structural modifications including: (1) an absence of a direct blood supply from th retinal circulation, (2) the presence of a which thins the retina locally, (3) a focal concentration of a class of colour sensitiv photoreceptors ('cones'), and (4) the inclusion of specialized circuitry which conserves the electrical responses of individual cones.

Few groups internationally are applying modern molecular tools to the investigati of primate retinal development and hum retinal disease. During 2001 we have developed new approaches to investigat the biology of the macula. Our focus is tc identify unique molecular profiles associated with the unique anatomy and physiology of the macula and fovea.

Projects in 2002:

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*: Cornish, Natoli, Chao, 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 specialization is lost and is associated with a decline in visual acuity. Our studies show that during development members of the Fibroblast Growth Factor family are expressed on cone photoreceptors in a pattern consistent with a role in cone differentiation. The results suggest that FGF signalling may be the mechanism through which cones obtain and maintain their elongated shape. The present focus is to block FGF signalling in retinas in culture and observe any related changes in cell morphology.



High powered microscopic view of the foveal region of the macula from SSI Retinal Development Group.

***Blood Vessel Growth During Foveal Development**: Allende, Sandercoe, Penfold, Provis.* Recently we have found that before the fovea forms the inner retina is hypoxic. In many conditions this hypoxia would signal the growth of blood vessels into the area; however, this part of the retina –the foveal region – remains avascular throughout life. The findings suggest that an unidientified factor inhibits growth of blodd vessels into this part of the retina. We are testing the hypothesis that transforming growth factor ß is highly expressed in this region and inhibits angiogenesis.

Macular Degeneration:

AMD occurring in Dry and Wet forms, involves disruption of the barriers which normally protect retinal tissue from outside influences. Blood-retinal barriers (BRB) exist at two principal sites; an inner barrier consisting of retinal vascular endothelial cells & glia limitans, and an outer barrier represented by a monolayer of retinal pigment epithelial cells.

Our studies aim to determine the distinguishing features of Wet and Dry AMD and provide an improved rationale for clinical management of the disease, for which treatment options are extremely limited; presently no recognised therapy is available for the Dry form.

Projects in 2002:

***Smoking & AMD:** Penfold, Provis, Billson.* We have reported, in an ABC Radio Health Report program, that AMD is causally linked to smoking. In the adult macula there is a critical balance between limited blood supply and high metabolic demand such that even minor perturbations of circulation, as may occur in incipient vascular disease or as a consequence of smoking, lead to metabolic stress in foveal neurons and/or glia. Such perturbations and the resultant physiological 'stress' may be the origin of signals which induce macular degeneration.

Previously SSI staff Norris Tsang, Philip Penfold, and Frank Billson looked at risk factors of macular degeneration, in particular the oxidative damage caused by smoking to the retina. Their investigation demonstrated that smokers were three times more likely to develop macular degeneration when compared to non smokers. This observation has been confirmed by the Blue Mountains Eye Study. The study estimates that approximately 100,000 people in Australia suffer late stage AMD. It was estimated that 20% of these cases were directly linked to smoking.

***Steroids & AMD:** Penfold, Wen, Madigan Provis.* A number of clinical pilot studies indicate that 'wet' retinal diseases, particularly AMD, may be treated by intravitreal administration of triamcinolone acetonide (TA), a corticosteroid. Although this class of drugs is known to display differential capacities to mediate anti-inflammatory and permeability effects, the modes of action of TA have not been defined.

Our studies suggest that TA has the capacity to modulate both the expression of adhesion molecules and cellular permeability of human epithelium. The results are consistent with clinical observations, indicating that reduction of the permeability of the outer BRB and down regulation of inflammatory stimuli are the principal effects of intravitreal TA *in vivo*. Intraocular fluid represents both a clinical indicator of barrier breakdown and a target for therapy.

***Neuronal transporters & glutamate homeostasis:** Penfold, Pow (Uni. of Queensland).* Exposure of isolated retinas to 30 micro molar D-aspartate, which is a substrate for all high affinity glutamate transporters, resulted in the accumulation of D-aspartate into Müller glial cells but not glutamatergic neurons as evidenced by immunocytochemistry for D-aspartate. Further incubation of such loaded retinas in physiological media, in the absence of D-aspartate, resulted in the slow release of accumulated D-aspartate from the Müller cells and its accumulation into populations of photoreceptors & bipolar cells.

This result indicates that after initial transport into Müller cells, reversal of direction of transport of D-aspartate, and thus by inference glutamate, by GLAST, readily occurs. D-aspartate released by Müller cells was strongly accumulated into cone photoreceptors which are known to express GLT-1, and into rod photoreceptors which we demonstrate here to express the retina specific glutamate transporter EAAT5 (excitatory amino transporter 5). Populations of glutamatergic bipolar cells, which express GLT-1 also exhibited avid uptake of D-aspartate. We conclude that the Müller cell glutamate transporter GLAST is responsible for most of the initial glutamate clearance in the retina after its release from neurones. However, some glutamate is also returned from Müller cells, to neurons expressing GLT-1 and EAAT5, albeit at a slow rate. These data suggest that the role of neuronal glutamate transporters in the retina may be to facilitate a slow process

of recycling glutamate back from Müller cells to neurons after its initial clearance from perisynaptic regions by GLAST.

Eye Cancers:

Retinoblastoma (Rb) and Uveal 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 and cell death 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 effects of therapies, particularly radiation, and the high incidence of metastases in melanoma

Projects in 2002

In vivo effects of sodium butyrate 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 efficacy of various retinoids and sodium butyrate in controlling intraocular tumor growth in a mouse model of Rb. These agents can induce tumour cell differentiation, and modulation of immune molecules and proteins involved in cell cycling and cell death. When used in combination with chemotherapy and radiation, some of these agents may reduce the dose of potentially cytotoxic therapies required when treating tumours.

Effects of radiation on Rb*: Madigan, Zhang & Stevens*. Rb cells are very sensitive to ionising radiation. Ongoing studies are using Rb and Rb-reconstituted cell lines to investigate some of the pathways important for Rb cell survival and death. For example, while most malignancies express a p53 gene mutation, both p53 (and p21 downstream) are upregulated following radiation of Rb cells, consistent with functional p53 in Rb.

Targeted radiotherapy of ocular melanoma*: Conway, Billson, Li, Lai, Madigan, Allen (St George Hospital, Uni. of NSW)*. Laboratory research has focused on targeting radiotherapy more precisely to melanoma cells to deal with both primary and metastatic disease. We have recently been studying a melanoma specific antigen (Mab 9.2.27) which may have potential as a target for radiotherapy treatment. Preliminary studies show that choroidal melanomas and choroidal melanoma cell lines (from Prof Jager, Netherlands)express 9.2.27 immunoreactivity.

A role for matrix metalloproteinases in uveal melanoma and retinoblastoma: *Lai, Conway, Billson, Provis, Crouch and Madigan*. 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. To date, the role(s) of MMPs ocular melanoma and particularly Rb have not been fully established. Our studies in ocular melanoma indicate that MMP-2 and MT1-MMP are involved in tumour angiogenesis. MMP-2 expression by fibroblasts within tumours may also implicate EMMPRIN, an inducer of MMP activity produced by neighbouring tumour cells, in melanoma growth.

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 Gilles, MB BS PhD FRACO, *Unit Head*
Florian Sutter, MD *Clinical Research Fellow*
Wei Luo, MD MPH, *Clinical Research Officer*
Meidong Zhu, MD PhD, *Clinical Research Officer*
William Chua, MB BS, FRACO, *Postgraduate student, Clinical Research*
David Van Reyk, BSc, PhD, *(Assoc. Lecturer, UTS), Research Associate*
Marina Tretiach, BAppSc, *Postgraduate student, Laboratory Research*
Svetlana Cherepanoff, BMed Sci MB BS, *Postgraduate student, Laboratory Research*
Goff Quin, MB BS, *Postgraduate student, Laboratory Research*
Jenny Wyndham, BSc Dip Ed MS MPH, *Postgraduate student, Laboratory Research*
Katherine Gill, BSc, PhD, *Laboratory Research Officer*

Research Activity

Clinical Group

The clinical group was established to test interventions developed by laboratory research, conducted by this and other groups within the Institute, by means of "randomised clinical trials" (RCT).

Projects in 2002

Intravitreal triamcinolone for diabetic macular oedema: *Sutter, Gillies, Simpson(Department of Public Health).* Following our successfully conducted RCT of an intravitreal injection of the steroid triamcinolone for "wet" age-related macular degeneration (AMD), we have commenced another RCT, funded by the Juvenile Diabetes Research Foundation, to test whether an injection into the eye of triamcinolone can reduce the risk of blindness in patients with diabetes and swelling of the central retina, or macular oedema. The short term results of this intervention are extremely promising, with a favourable response in around 60% of eyes that would otherwise likely go blind since they have failed laser treatment. We will need to also check the long-term (2 year) results before we can recommend this treatment for routine use.

Retinal bypass study: *McAllister (University of Western Australia), Gillies, Mitchell.* This is an NHMRC supported randomised clinical trial of a "retinal vein bypass" procedure using a high powered laser for central retinal vein occlusion, a common, often devastating condition. It is being undertaken in collaboration with Dr. Ian McAllister of Lions Eye Institute, WA.

Photographic outcomes in the intravitreal triamcinolonefor wet AMD study: *Chua, Gillies.* In order to understand how intravitreal triamcinolone works, the retinal angiograms and colour photographs of the macula were analysed with respect to the nature & size of the lesions & their changes in both size & leak after treatment.

Collaboration in international randomized clinical trials: The unit has participated as one of the treatment centers in major international studies sponsored by EYETECH Pharmaceuticals of a new drug which is directed at "Vascular Endothelial Growth Factor", a protein that is thought to play a leading role in the development of macular oedema and wet AMD. As well as generating much needed funds for supporting our basic research activities, the Unit's participation in such studies allow patients of the Institute and the Sydney Eye Hospital access to the latest treatments available anywhere in the world.

Laboratory Group

Retinal vascular diseases caused by abnormal leakage of fluid through small blood vessel walls (capillaries) are among the commonest causes of blindness – especially in diabetic retinopathy, retinal vein occlusion, ocular inflammation and the wet form of AMD. To investigate the molecular basis of these diseases the group has developed laboratory (in vitro) and animal (in vivo) models to study retinal vascular permeability.

Projects in 2002

The effect of paravascular cells on retinal vascular 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 the laboratory model of retinal vascular permeability that we have developed. In addition to assessing the effects of these cells on vascular permeability, the effects of various conditions that mimic human disease such as low oxygen levels have also been assessed.

The effect of retinal laser treatment on the permeability of retinal vessels: *Quin, Gillies, Tretiach.* 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 characterized to explore the effects of retinal laser treatment more fully. The identification of a barrier-restoring factor may have therapeutic potential.

The role of oxidative damage in diabetic retinopathy: *van Reyk, Gill, Gillies*. Whilst it is suspected that oxidative stress may contribute to diabetic retinopathy, the identification of which antioxidants might be useful as a potential 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. We are using high performance liquid chromatography to detect the target and 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 the retina are known to occur in 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, Wakefield (Dept Pathology, University of NSW), DiGirolamo (Dept Pathology, University of NSW)*. MMPs are a group of enzymes that can digest extracellular materials as well as cell surface receptors. The presence of MMPs was characterized 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.

Tyrosine phosphorylation of junctional complex proteins: *Gillies*. We have proposed that chemical modification loosens the junctions holding vascular cells together resulting in increased leak. Increased leak was induced by the cytokines VEGF and TGF beta and was found to be correlated with increased phosphorylation at zones of cell-cell contact. Immuno-precipitation studies suggest that the phosphorylated protein is VE-Cadherin. If these changes can be inhibited by tyrosine kinase inhibitors, it may be that we have identified another new potential approach to the treatment of retinal swelling.

Retinal Dystrophy Research Group

The Retinal Dystrophy Research Group led by Professor Jonathan Stone, based in the Department of Anatomy and Histology, recently joined the SSI and commenced a collaborative project at the Sydney Hospital and Eye Hospital campus. The research program of this group is summarised below. Clearly there are many areas of common interest both in laboratory and clinical research and it is planned to expand the presence and activity of this group at the SSI in 2003 in preparation for an NHMRC program grant application.

Group Members

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

Research Program

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

- The group have made a series of findings novel in the understanding of photoreceptor death, in a range of rodent models, and in the human.
- The group have made a series of findings novel in the understanding of photoreceptor survival.
- The group have developed new techniques and collaborations, to take the analysis of the mechanisms of photoreceptor disease to the molecular level, and to include epidemiology of human disease.
- The group have begun the organisation of clinical trials, to deploy some of this knowledge.

The collaborative research project commenced in the SSI in 2002 is summarised below.

Oxygen-regulated genes in photoreceptor death: The RP1 gene: *Geller, Provis*. Following the publication by others of the sequence of the retinitis pigmentosa (RP1) gene, and identification of multiple mutations that cause a dominant form of retinitis pigmentosa, riboprobes have been

constructed for RP1 in 5 species (rat, mouse, cat, rabbit, human). The probes label the photoreceptors specifically, and are being used to monitor the regulation of RP1 expression in several models of retinal degeneration. Similarly, clones of the hypoxia inducible factor-1 alpha were isolated and used as templates for synthesising riboprobes, allowing for analysis of a second oxygen regulated gene in animal models such as light damage, retinal detachment, and the rd mouse. This work has extended our molecular biological tools for the study of the mechanism of the effects of oxygen on photoreceptor death and survival.