

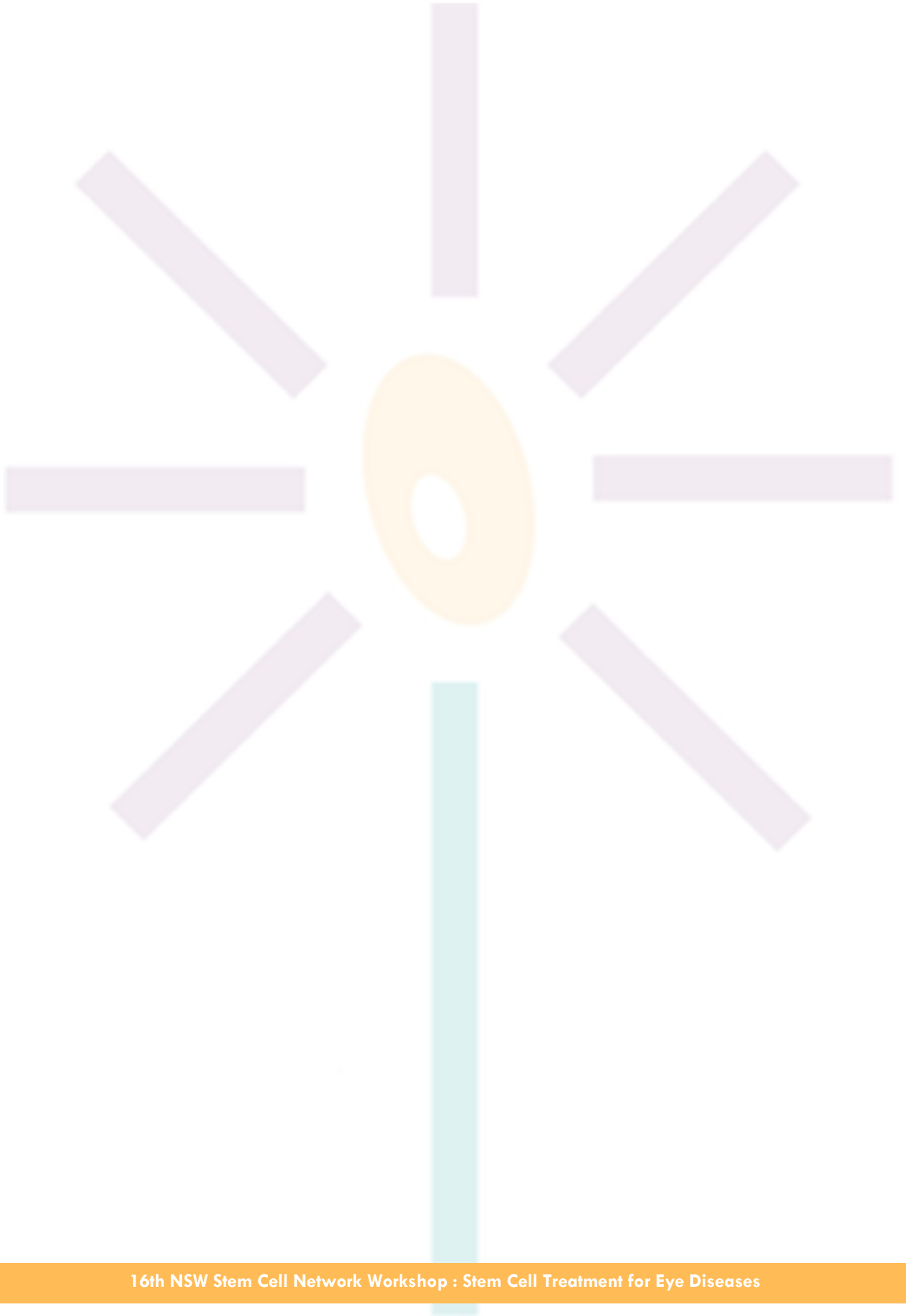


16th NSW Stem Cell Network Workshop Stem Cell Treatment of Eye Diseases

School of Medicine,
University of Western Sydney,
Campbelltown Campus
16th April, 2012

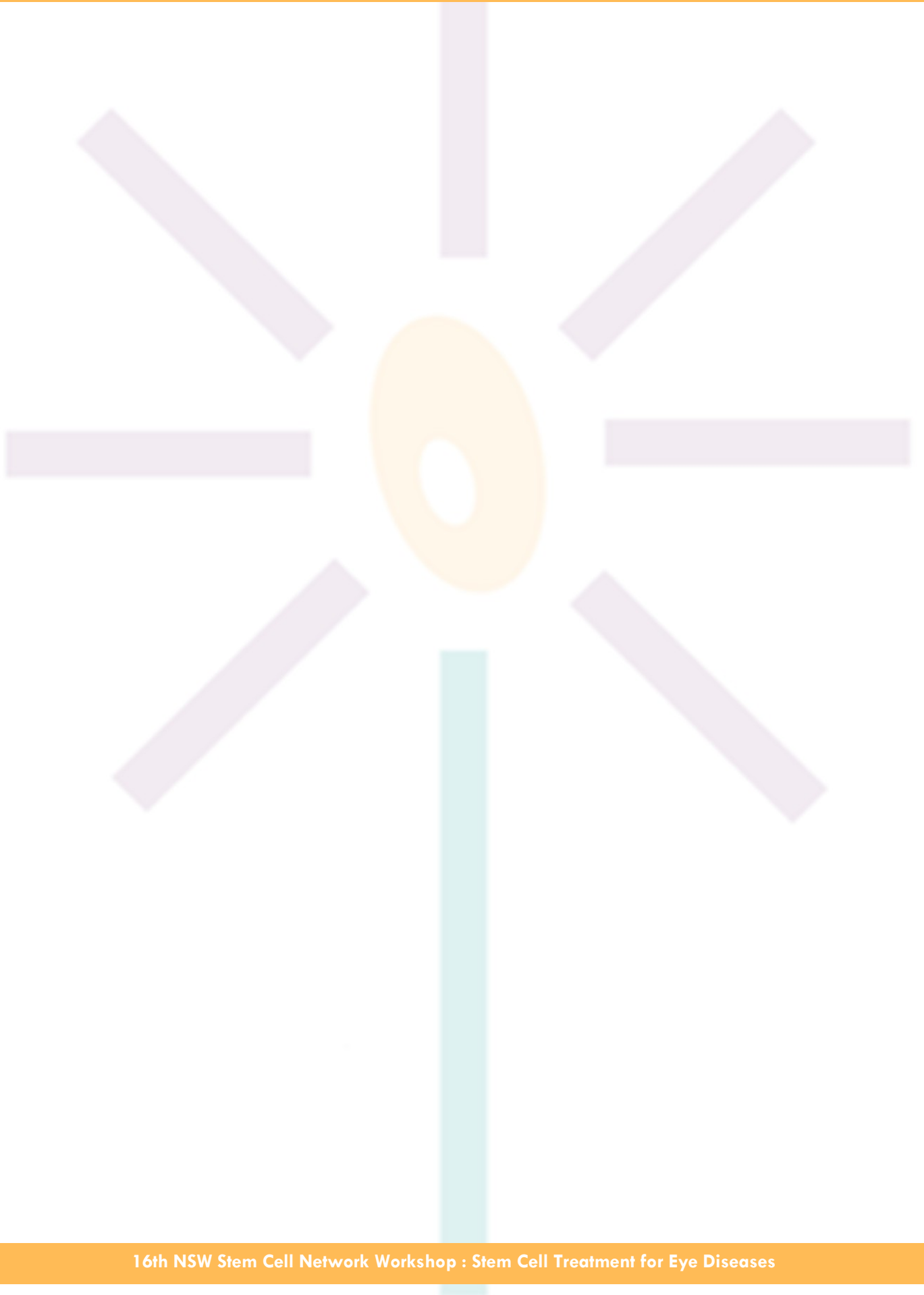


Wednesday, 31st August, 2011. Darlington Centre Conference Room, 174 City Road, Darlington





NSW Stem Cell Network



Welcome to the 16th Workshop of the NSW Stem Cell Network.

It has been 10 years since the NSW Stem Cell Network came into being as a result of a desire by stem cell professionals to network. Its initiation was stimulated by the creation of the 1st human embryonic stem cell line just a few years previously, in Wisconsin.

The Workshop has been the key strategy used to network professionals, this one being the 16th of these.

The theme of today's workshop is *Stem Cell Treatment for Eye Diseases*. It is fitting that eyes should be the centre point for today as it was only a few months ago that the first positive outcome from the transplantation of pluripotent stem cells into people was announced, for the treatment of macular degeneration. Admittedly, the results announced were only preliminary, but importantly they begin to translate the hope of stem cells and into reality.

A second benefit occurred a year ago with a publication from the RIKEN group showing how stem cells could be used to begin to create the eye itself.

Both of these areas will be covered in the Workshop, by Professors David Hinton and Mototsugu Eiraku from California and Japan respectively.

The Network is grateful to Dr Michael O'Connor from the University of Western Sydney who has championed the idea of this workshop. He has been a strong driving force for it, ably supported by Ms Sarah Mustafa from the Network.

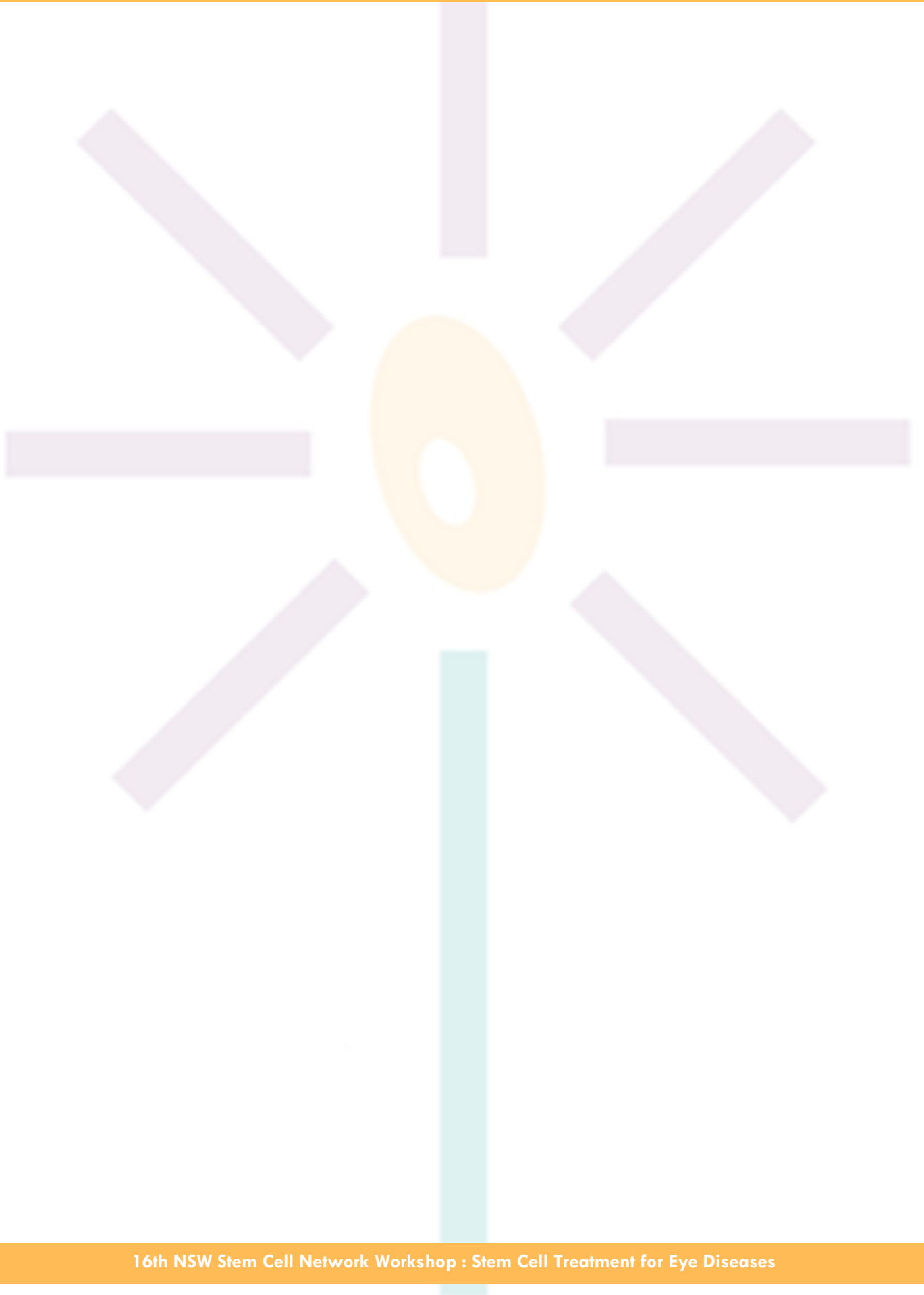
We are also grateful to our many sponsors, the University of Western Sydney for allowing the workshop to be held on its premises, and the Office for Medical Research in the NSW Department of Health for its ongoing support.

Enjoy the Workshop and may you be stimulated by it.

Bernie Tuch

Director, NSW Stem Cell Network

NSW Stem Cell Network



Glimpsing the future.

Dr. Michael D. O'Connor

Research Lecturer in Regenerative Medicine

School of Medicine, University of Western Sydney

Nations around the globe face ever-increasing social and financial costs from diseases related to aging. In Australia the direct and associated costs of only two such conditions, cataract and macular degeneration, are currently estimated at over \$3 billion annually^{1,2}; a figure expected to double in the next 10 to 15 years³. For the majority of macular degeneration patients there are no treatment options. While cataract can be effectively treated surgically, issues of secondary cataract and accommodation loss are incompletely resolved. Novel strategies are therefore needed to provide new or improved therapies for these and other eye diseases or injury⁴.

Stem cells hold great potential for novel ocular therapies due to two defining features: their capacity to generate functional human cell types (via differentiation) for research or transplantation; and their ability to make copies of themselves (via cell division) while retaining the ability to differentiate. Embryonic and induced pluripotent stem cells can produce any cell in the body and are already being applied clinically. For example, phase I/II clinical trials using retinal pigment epithelial cells made from human embryonic stem cells are proving safe and able to improve vision in age-related macular degeneration and Star-gardt's macular dystrophy patients⁵. Additionally, while tissue-specific (or 'adult') stem cells typically only produce cells from the tissue in which they are found, they too have applications for ocular therapy. For example, adult limbal stem cells have successfully been used to repair corneal disease and injury⁶.

Cutting edge stem cell eye research will soon be discussed in detail at the 16th NSW Stem Cell Network meeting to be held April 16th at the School of Medicine, University of Western Sydney. This meeting, titled "Stem Cell Treatments for Eye Diseases", will feature leading stem cell researchers from Australia, Japan and the USA. The presentations will include research on the cornea, lens and retina, and will cover issues such as: use of stem cells to understand genetic control of eye development; stem cell harvesting, culture, and drug screening; as well as stem cell transplantation therapies. While much work remains to be done, these advances and the prospect of more to come bring to mind Alfred Tennyson's words: "For I dipped into the future, far as human eye could see, Saw the Vision of the world, and all the wonder that would be". For developing stem cell treatments to debilitating eye disease and injury, it appears the beginning of this future is upon us.

For more information or to register for the conference, contact the NSW Stem Cell Network on (02) 9552 9981 or by email at s.mustafa@stemcellnetwork.org.au.

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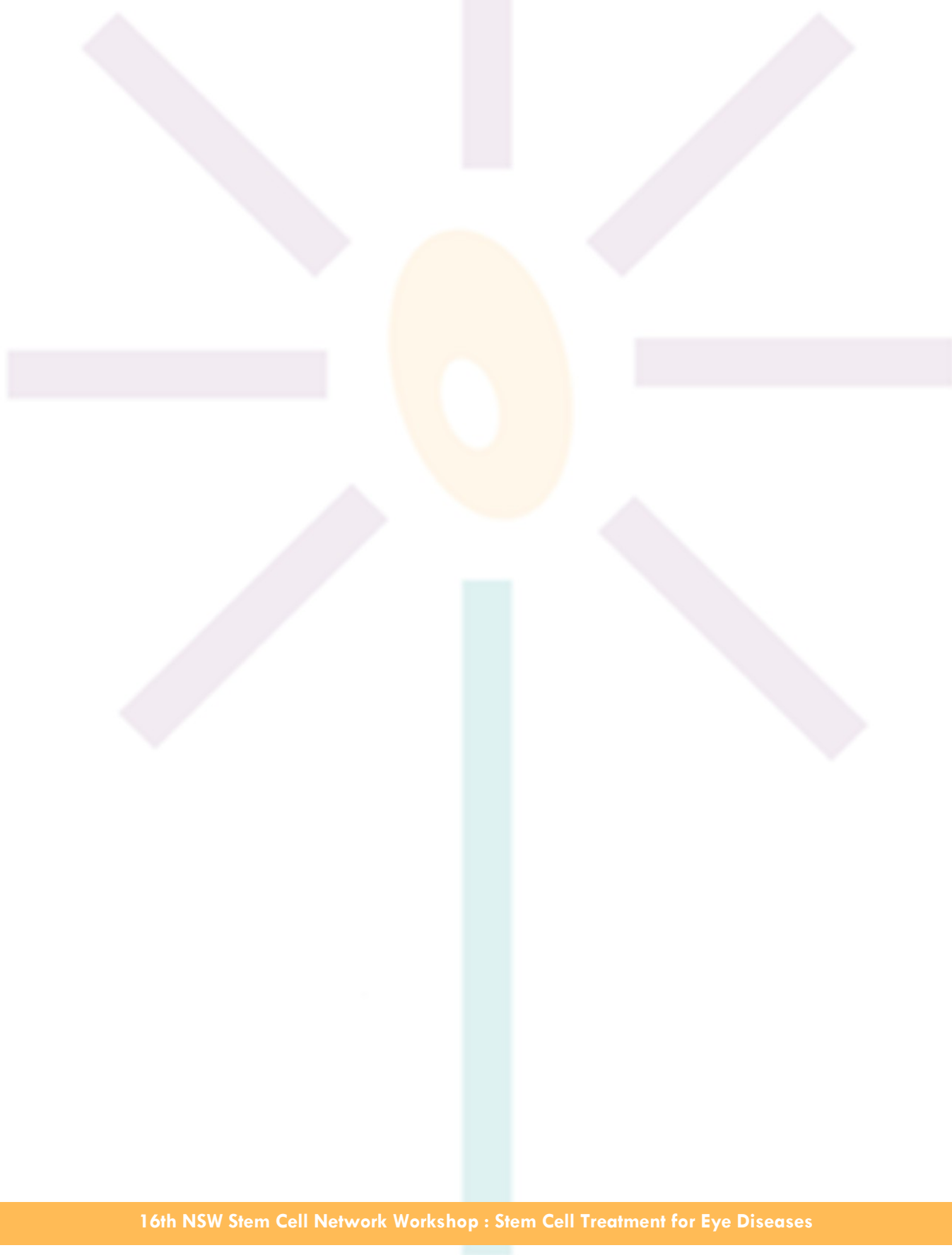
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Program

9:30am	Registration opens / Light Refreshment
10:00am	Opening Address: Prof. Annemarie Hennessy Dean of School of Medicine, University of Western Sydney
Session 1 Chair:	Stem cells as tools to understand ocular development Dr Mark Hill – University of New South Wales
10:10am	Dr. Mototsugu Eiraku - Centre for Developmental Biology, RIKEN Self-organized formation of optic cup in vitro
10:50am	Prof. Roger Truscott – University of Sydney Getting old and going blind
11:15am	Morning Tea / Networking
11:35am	Dr. Michael O'Connor – University of Western Sydney Modelling lens & RPE development with stem cells
12 noon	A/Prof. Robyn Jamieson – University of Sydney Genetic Eye Diseases and Potential for Stem Cell Therapy
12:25pm	Lunch / Networking
Session 2 Chair:	Clinical cell transplantation and biocompatible ocular surfaces Prof. John Morley – University of Western Sydney
1:25pm	Prof. David Hinton - University of Southern California, USA Human ES cell-derived RPE for therapy of macular degeneration
2:05pm	A/Prof. Nick Di Girolomo - University of New South Wales Corneal Stem Cell Support Factors: Vitronectin and its Receptor $\alpha v \beta 5$
2:30pm	20 mins break
2.50pm	A/Prof. Mark Daniell - University of Melbourne Stem Cell Transfer Technology – From Test Tube to the Eye
3:15pm	Prof. Traian Chirila - Queensland Eye Institute Silk fibroin as Substratum for Corneal and Retinal Cells
3:40pm	Announcing poster session winners Refreshments / Networking

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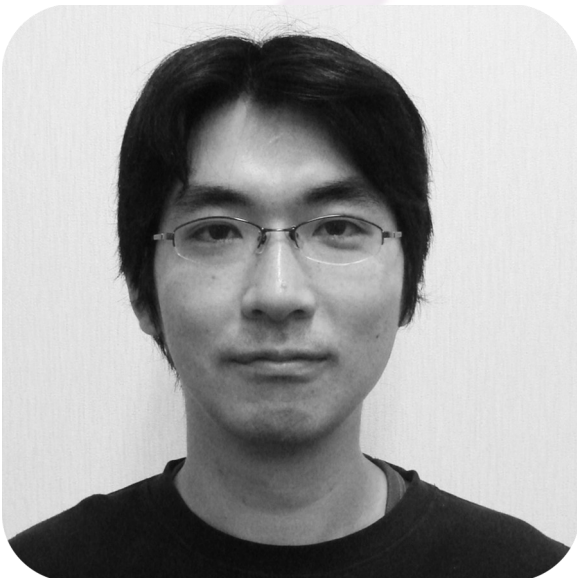


Plenary Speaker: Dr Mototsugu Eiraku - Centre for Developmental Biology, RIKEN

Dr. Mototsugu Eiraku - Centre for Developmental Biology, RIKEN, Japan

Self-organized formation of optic cup in vitro

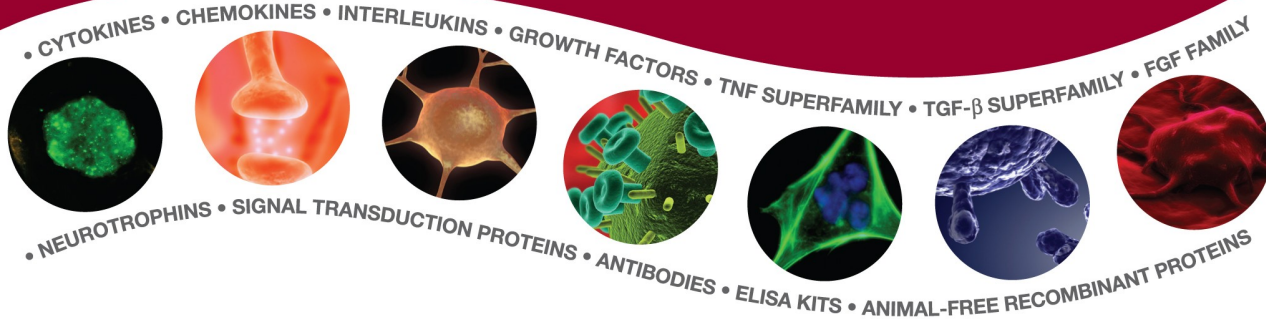
The balanced generation of organ requires the orchestration of multiple cellular interactions to create the collective cell behaviors that progressively shape developing tissues. In vitro generation of compound tissues with complex structures is a major challenge in cell biology. We report the dynamic, autonomous formation of the optic cup (retinal primordium) structure from a three-dimensional culture of mouse ES cell aggregates. ES cell-derived retinal epithelium spontaneously formed hemispherical epithelial vesicles that become patterned along its proximal-distal axis. While the rigid proximal portion differentiated into pigment epithelium, the flexible distal portion progressively folded inward to form a shape reminiscent of the embryonic optic cup. In long-term culture, the ESC-derived optic cup generates a fully stratified retinal tissue consisting of all major neural retina components. We demonstrate that optic-cup morphogenesis in this simple cell culture depends on an intrinsic self-organizing program involving stepwise and domain-specific regulations in local epithelial properties.



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Speaker: Prof. Roger Truscott - University of Sydney

Prof. Roger Truscott - University of Sydney

Getting old and going blind

The discovery that the primary reason for human age-related lens disorders can be traced to the decomposition of long-lived macromolecules has opened up a number of avenues for research. One major thrust involves the use of the lens as a model system for elucidating the processes involved in the deterioration of such ancient macromolecules. The human lens is ideal for this purpose because it is composed of just a few major proteins (the crystallins) and samples of proteins of different ages can be readily obtained from eye banks. One key question is this: what are the predominant processes responsible for protein degradation over long periods of time? For example, is racemisation, deamidation, modification by reactive molecules, or peptide bond cleavage of most importance for inducing protein denaturation? Do the time scales for each of these events differ? Which amino acids are involved? Are some proteins, and certain regions within individual proteins, more susceptible? Uncovering the answers to these questions has obvious relevance for the aged human lens, however its impact may be more widespread since old proteins are found in a number of tissues in the body.

Understanding these processes will provide fundamental information on an important component of human aging and may even allow us to intervene in some aspects, in order to minimize the impact of protein degeneration on human health.

Professor Roger Truscott is an NHMRC Senior Research Fellow at the Illawarra Health and Medical Research Institute. Until recently, his group was based at the Save Sight Institute, Sydney Eye Hospital. He is interested in human aging and in particular conditions of the lens associated with old age, such as presbyopia and cataract. Roger obtained his PhD in biochemistry from Melbourne University and after a postdoctoral fellowship at the Children's Hospital Melbourne was awarded a Fulbright fellowship to Stanford University. He lectured at Wollongong University from 1985 till 2005 when he moved to Sydney University.



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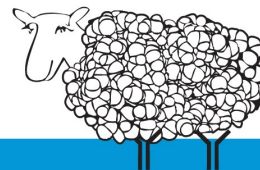
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Speaker: Dr. Michael O'Connor - University of Western Sydney

Dr Michael O'Connor - University of Western Sydney

Modelling lens & RPE development with stem cells

Cataract and macular degeneration account for two-thirds of Australians with low vision or blindness that cannot be improved by refractive correction. Together, these diseases cost the Australian economy approximately \$3 billion annually. A key risk factor for both diseases is old age and, due to population aging, their incidence and costs are increasing.

Access to adequate amounts of normal and diseased human lens and retinal tissue has been one factor limiting our ability to develop novel treatments for both cataract and macular degeneration. Recent advances in human pluripotent stem cell differentiation techniques have provided rudimentary methods for producing large amounts of normal and disease-specific lens epithelial cells and retinal pigment epithelial cells. The UWS Regenerative Medicine group is optimising these methods and using them to better understand normal and diseased lens and retinal development, with the aim of developing novel cataract and macular degeneration treatments via drug screening and cell replacement therapy.



Dr. Michael O'Connor is a Research Lecturer in the School of Medicine at the University of Western Sydney. He obtained his PhD from the University of Sydney in 2005, creating an animal-based culture system that can regenerate functional ocular lenses in vitro. Upon completing his PhD Michael undertook postdoctoral studies with Prof. Connie Eaves in Vancouver, Canada, where he identified new genes that are required to maintain human pluripotent stem cells. During this time he was also closely involved in the commercial development of the feeder-independent pluripotent stem cell media, mTeSR®1 and TeSR™2. Michael's current research uses human pluripotent cells to learn more about normal and pathological eye development.



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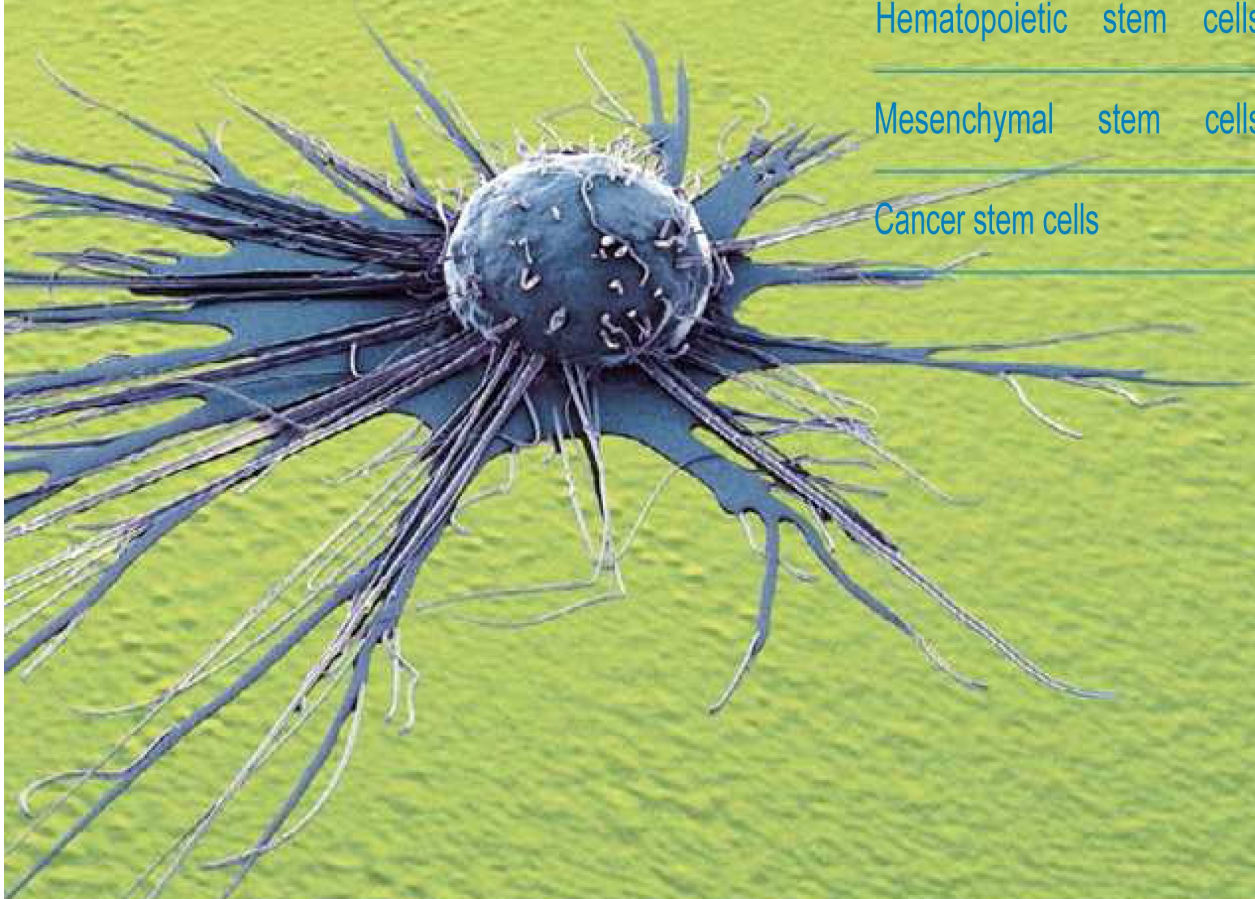
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Speaker: A/Prof. Robyn Jamieson - University of Sydney

A/Prof. Robyn Jamieson – University of Sydney

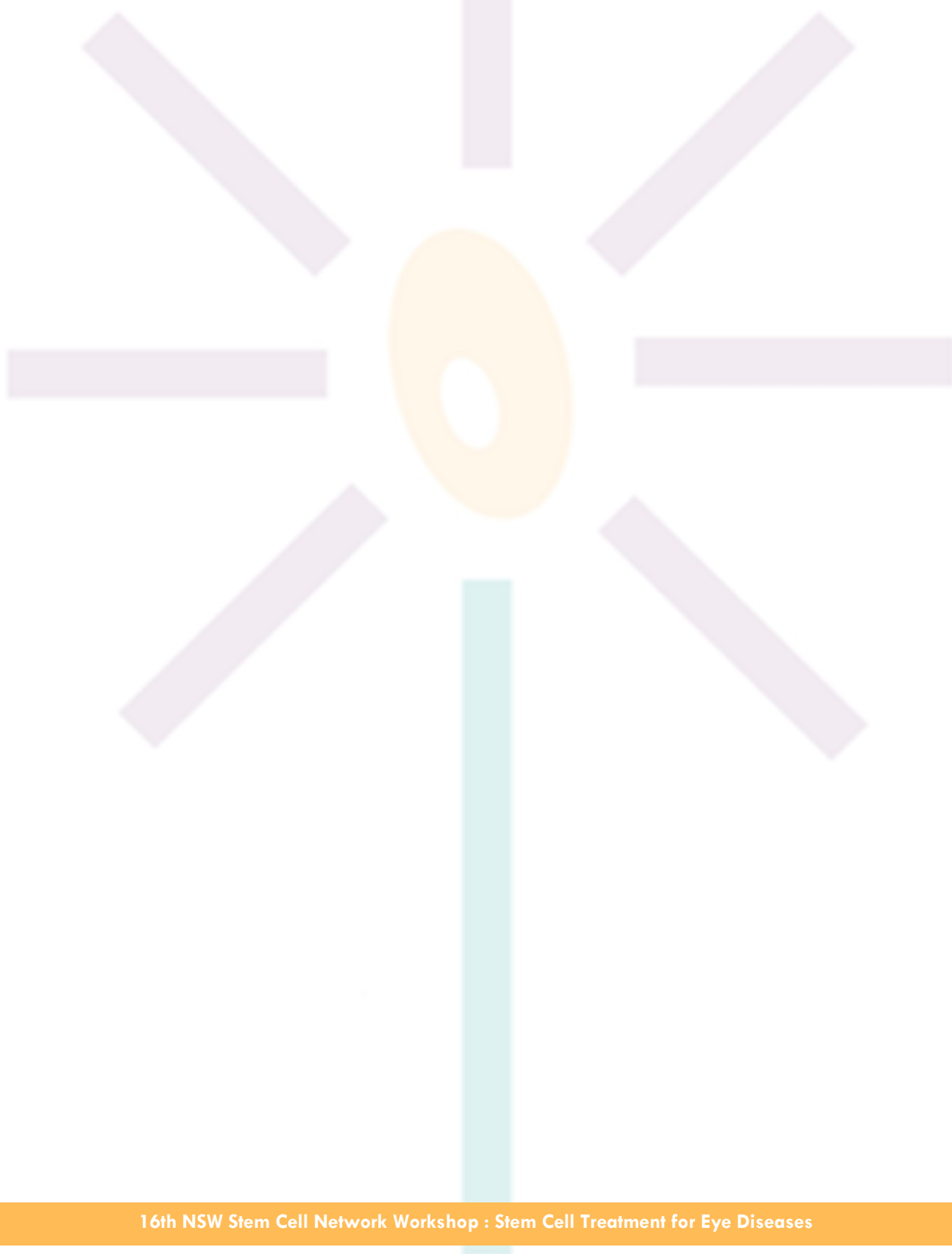
Genetic eye diseases and potential for stem cell therapy

There are no or limited treatment options for many genetic eye disorders including retinal and corneal diseases and those leading to glaucoma. This presentation will focus particularly on the inherited retinal dystrophies, a clinically heterogeneous group of progressive disorders that primarily affect the function of the photoreceptors and the RPE. There are at least 50 disease genes that can lead to retinal dystrophies due to defects in processes including phototransduction and ciliary transport in the photoreceptors. Genetic abnormalities of the function of the RPE can also lead to retinal degeneration. In the retinal degenerative disease of retinitis pigmentosa, rods are predominantly affected leading to night blindness and tunnel vision. Later in the disease, cones are also affected and there may be complete blindness. Stem cell therapies that may be considered include retinal neuroprotection and retinal neuroregeneration. In a form of retinitis pigmentosa where the ability of the RPE cells to phagocytose photoreceptor outer segments is impaired, subretinal transplantation of healthy RPE cells generated from ES cells has demonstrated photoreceptor protection with preservation of visual function in a rat model. Neuroregenerative applications are under active exploration since they hold the hope of reversing functional loss. Investigations in mouse models of genetic degenerative retinal diseases affecting photoreceptors, have shown that immature retinal cells generated from ES cells can integrate into the retina after subretinal transplantation. Transplanted cells exhibit differentiated photoreceptor features with improved functional light responses, however there are several hurdles in such treatments including generation of the appropriate highly ordered connections of the retinal network. Stem cell approaches offer the possibility of neuroprotective and neuroregenerative therapies in genetic retinal diseases where treatment options are currently absent or limited.

Associate Professor Robyn Jamieson heads the Eye Genetics Research Group at The Children's Hospital at Westmead, the Children's Medical Research Institute, and the Save Sight Institute, University of Sydney. She is also a Clinical Geneticist at The Children's Hospital at Westmead, and Westmead Adult Hospital, Sydney, specialising in genetic eye disorders. As an NHMRC scholar, she undertook PhD studies in developmental biology at the University of Sydney. Postdoctoral studies in genomics and molecular genetics were undertaken at the University of Manchester, UK. Her work in molecular genetics and genomics related to human patients, in combination with mouse model studies, has been integral in the understanding of a number of novel genes in eye disease. Her work includes application of next-generation sequencing strategies and functional studies for novel disease gene identification and characterisation. Identification of the disease genes directly indicates critical pathways in development, differentiation and maintenance of normal vision, and provides direction for development of improved treatments for these conditions.



NSW Stem Cell Network



Plenary Speaker: Prof. David Hinton - University of Southern California USA

Prof. David Hinton - University of Southern California, USA

Human ES cell-derived RPE for therapy of macular degeneration

Clinical trials using human embryonic stem cell (hESC) derived retinal pigment epithelial cells (RPE) are ongoing in the USA for Stargardt's disease and the atrophic form of age related macular degeneration (AMD). The RPE is a major site of pathology in both the neovascular and non-neovascular forms of AMD and reproducible differentiation of RPE cells from hESC has been achieved in several labs including our own at the Broad CIRM Center for Regenerative Medicine and Stem Cell Research at USC. Cell suspensions of hESC-RPE have shown safety and efficacy in animal models of retinal degeneration. While current FDA approved trials are using subretinal injections of cell suspensions, we propose use of hESC-derived RPE grown as highly polarized monolayers with morphological, molecular and functional characteristics of the normal RPE layer. These cells are grown on non-biodegradable substrates for subretinal delivery. Polarized hESC-derived RPE monolayers demonstrate very high levels of apical secretion of pigment epithelium derived growth factor which may provide additional neuroprotective effects. The immune privilege of the subretinal space provides an environment that may limit the need for systemic immunosuppression. Preclinical studies in the Royal College of Surgeons rat demonstrate the efficacy of this therapy as measured by histology and tests of visual function.

David R.Hinton MD is a board certified Pathologist (Neuropathology) with over 20 years experience evaluating the pathogenesis of blinding retinal disorders with a focus on age related macular degeneration (AMD). He is the Gavin S. Herbert Professor of Retinal Research and Professor of Pathology and Ophthalmology of the Keck School of Medicine of the University of Southern California and is Vice Chair of the Department of Pathology. Dr. Hinton has been designated a Master Teacher at the Keck School of Medicine. He is a founding member of the Beckman Macular Research Center at the Doheny Eye Institute. He is widely known for his expertise in the biology of the retinal pigment epithelium (RPE) and in the evaluation of RPE in animal models of retinal disease. He has





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Speaker: A/Prof. Nick Di Girolamo—University of New South Wales

A/Prof. Nick Di Girolamo - University of New South Wales

Corneal Stem Cell Support Factors: Vitronectin and its Receptor $\alpha\text{v}\beta 5$

Purpose: Limbal stem cell deficiency (LSCD) is a blinding corneal condition treatable through expensive, time consuming, and complicated autologous or allogeneic stem cell (SC) transplantation. If corneo-limbal SCs (LSCs) can be more readily identified, isolated, and maintained *ex vivo*, better quality and more stable grafts could be prepared for patients suffering this debilitating disease. We postulate that the extracellular matrix (ECM) glycoprotein, vitronectin (VN) and its integrin receptor $\alpha\text{v}\beta 5$ play an integral role in supporting LSCs within their niche microenvironment.

Methods: Histological and immunolocalization studies were conducted on cadaveric human fetal and adult ocular tissues. Limbal epithelial cells were expanded from donor human corneal rims using a tissue explant technique or enzymatic dissociation and $\alpha\text{v}\beta 5$ expression determined by flow cytometry. Integrin $\alpha\text{v}\beta 5^{+/-}$ cells were further selected using either magnetic microbeads or FACS sorting. Functional colony forming assays, and immunocytological and RT-PCR assays for SC marker expression were conducted on fractionated cells.

Results: VN was identified as an ECM component on the limbal basement membrane, and was restricted to SC-like clusters within the limbal region, but was not expressed in the differentiated compartment of the central cornea. Furthermore, its receptor $\alpha\text{v}\beta 5$ -integrin co-localized to N-cadherin $^{+}$ /CK-15 $^{+}$ putative LSCs. $\alpha\text{v}\beta 5$ expression was restricted to less than 5% of total isolated limbal epithelial cells and was used to enrich for a population of presumed LSCs based on functional colony forming capacity and enhanced expression of LSC-associated genes.

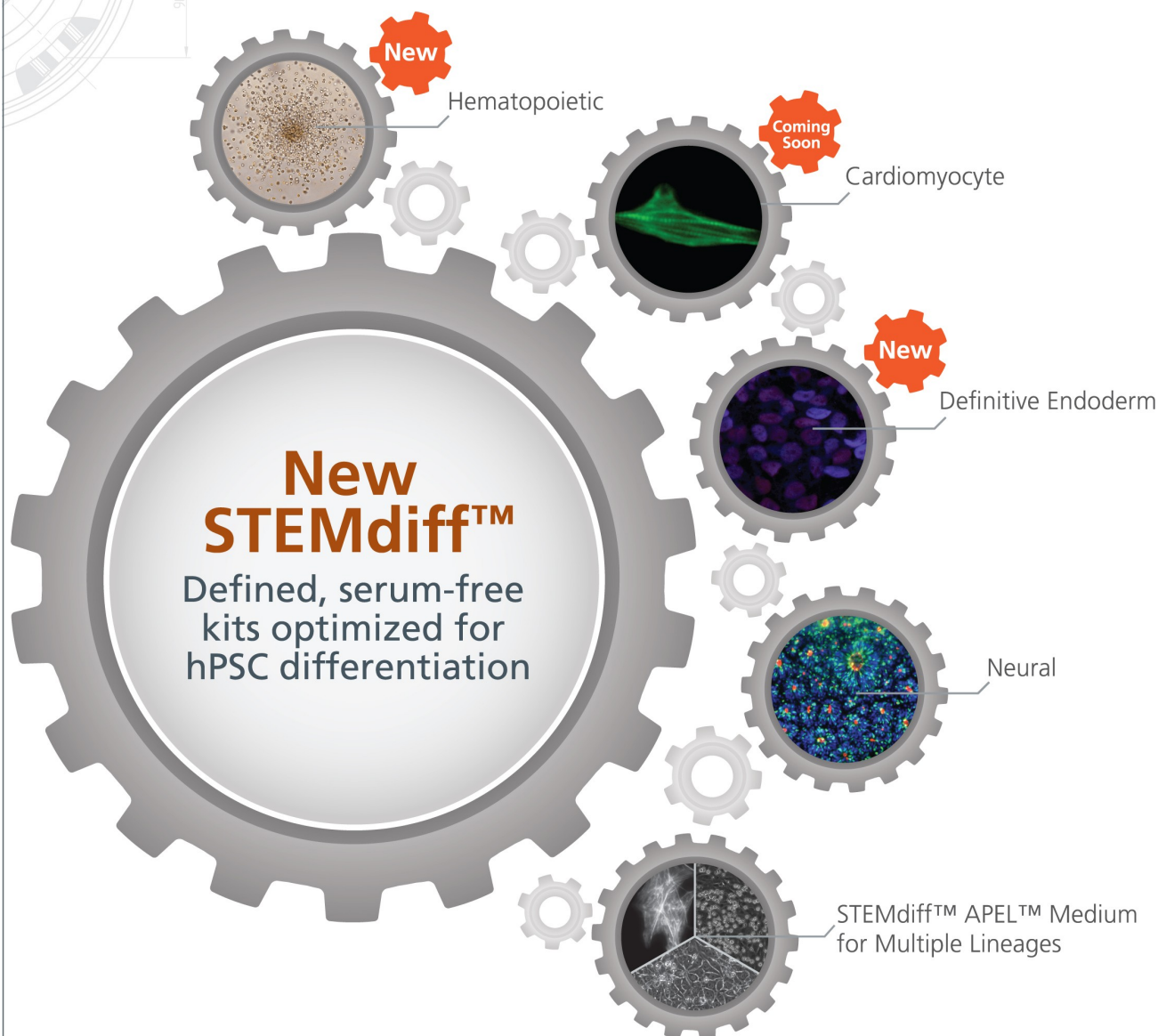
Conclusions: Our tissue-based studies indicate that a VN/ $\alpha\text{v}\beta 5$ axis plays an important role in maintaining LSCs within their niche. Our *in vitro* data supports this concept; however until the precise proteomic profile of the niche is disclosed, it may be difficult to establish the ideal culture conditions to nurture these cells long-term. Due to the unique, transparent and accessible nature of the human limbus and its SC reservoir, information gained from these studies may inform researchers developing treatment strategies for other human organ systems.



A/Professor Nick Di Girolamo leads the Ocular Diseases Research Group at the University of New South Wales as an independent researcher with a research program that directly impacts on patient's care. His team developed a novel cell replacement strategy which utilizes autologous corneal stem cells grown on a contact lens for patients with blinding corneal disease.

He has >70 publications, 16 appear in the lead experimental ophthalmology journal *IOVS* indicating he is consistently publishing in the highest ranked journals in the field. His work has been cited >1700 times and his h-index is 25. His research has been funded through peer reviewed national and institutional grants and philanthropic donations equating to over \$3M. He was awarded category winner of the ABC's New Inventors program for 2009 for his invention on growing stem cells on contact lenses as a means of transplanting them into patients with blinding corneal disease. He was a Finalist, for the UNSW Inventor of the Year 2010.

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Speaker: Mark Daniells - University of Melbourne

A/Prof. Mark Daniell - University of Melbourne

Stem Cell Transfer Technology – From Test Tube to the Eye

An unresolved issue in stem cell transplantation is the ideal method to transfer cultivated cells from the culture plate to the eye

We have developed novel methods of cell transfer.

1. Plasma polymer coated contact lenses for external use.
2. Biodegradable membrane for endothelial transfer

Results: Potential plasma polymer coatings were tested in vitro and in vivo to determine optimum conditions for cell proliferation, differentiation and transfer.

The optimum coating is now being used in a clinical trial.

A PEG Hydrogel was constructed that provided a substrate for endothelial cell growth, was biocompatible to the eye, biodegradable, transparent and robust enough to withstand surgical implantation. Cell culture conditions were optimised for maximal growth.

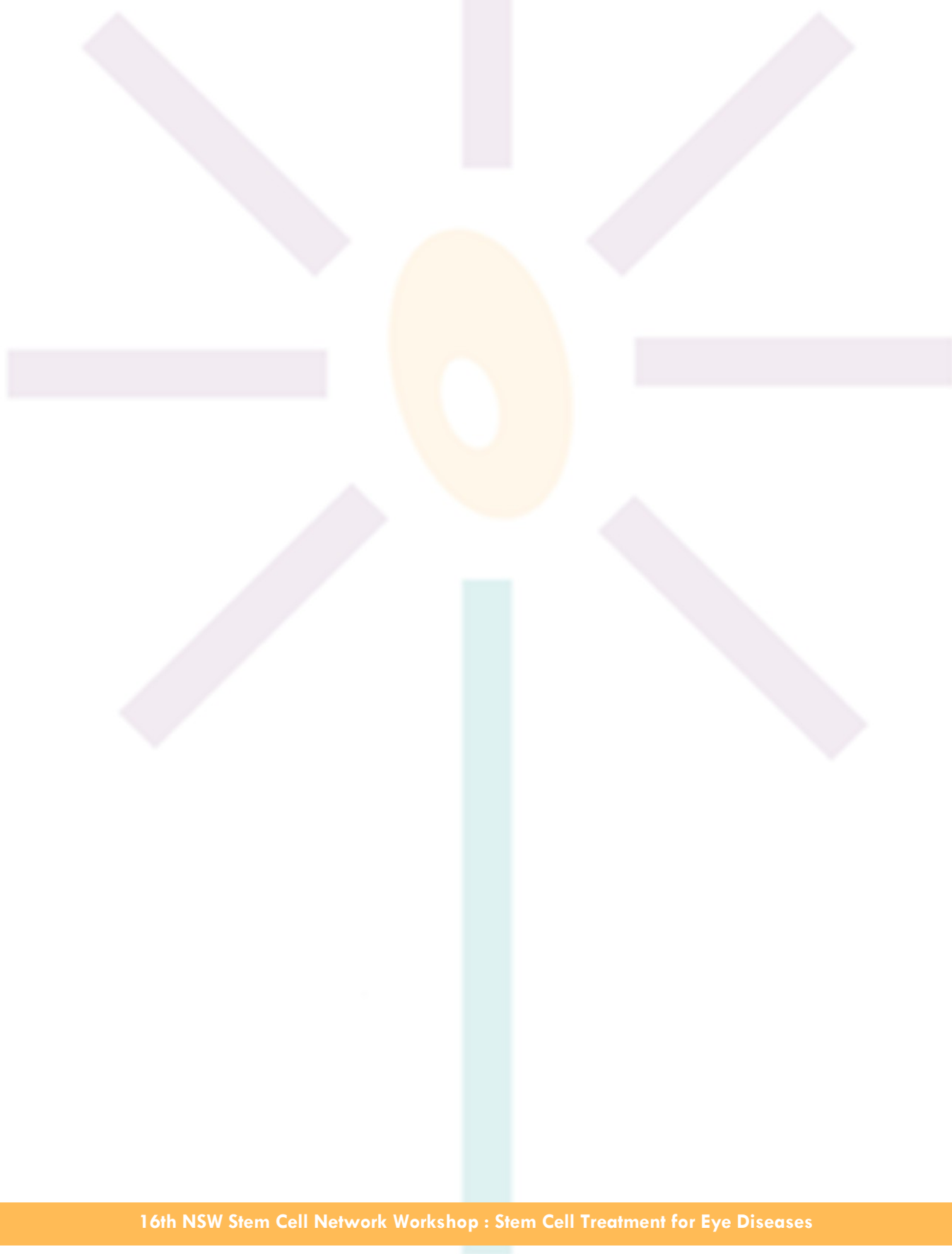
Conclusion: Plasma polymer coated contact lenses are a suitable vehicle for delivery of cells to the ocular surface. Clinical trials have commenced.

PEG-Hydrogel sheet designed for endothelial cell transfer has been developed. Mock DSEK surgery in sheep is the next step.



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Speaker: Prof. Traian Chirila - Queensland Eye Institute

Prof. Traian Chirila - Queensland Eye Institute

Silk fibroin as Substratum for Corneal and Retinal Cells

Fibroin is the main protein component of silks, where is accompanied by another protein, sericin. There is an established methodology to isolate fibroin from the silk produced by the domesticated silkworm (*Bombyx mori*). Fibroin presents characteristics suitable for a biomaterial for tissue engineering applications. Being biocompatible, transparent and slowly biodegradable, *Bombyx mori* silk fibroin (henceforth BMSF) appears as especially appropriate for the regeneration of ocular tissues.

Our work over the last five years, described in 12 papers published so far, showed that BMSF membranes can function as substrata for the attachment and proliferation of various types of ocular cells, including corneal limbal epithelial cells (presumed stem cells or progenitor cells), limbal mesenchymal stromal cells, corneal endothelial cells and retinal pigment epithelial (RPE) cells, either commercial or primary lines. Possibility to grow these cells on a substratum is of significance in the creation of tissue-engineered constructs for the restoration of ocular surface, for endothelial keratoplasty or for the transplantation of retinal cells in the management of age-related macular degeneration.

While our results with BMSF were promising, an enhanced cellular adhesion would be even more beneficial. BMSF does not contain in its structure recognition peptide ligand sequences (such as RGD, YIGSR, DGEA etc.) for integrins, the main receptors mediating cell anchorage to substrata. As the wild silkworm (*Antheraea pernyi*) fibroin (APSF) contains naturally RGD sequences, we directed our attention to this type of fibroin, and developed a method to isolate APSF. Our efforts are now focused toward improvement of the isolation method of APSF and its evaluation as substratum in engineered constructs for ocular regeneration.



Traian V. Chirila was born and educated in Romania, where he obtained a BEng in polymer technology and a PhD in organic chemistry (1981). After ten years of research in polymers and organic chemistry, he relocated to Australia as a political refugee. During 1984 he was a Research Fellow at the School of Applied Chemistry of Curtin University of Technology. In 1986 he joined Lions Eye Institute in Perth as a Senior Scientist with the task of establishing a department for research and development of polymeric biomaterials for ophthalmology. He was made a Fellow of Royal Australian Chemical Institute (RACI) in 1992 and received the RACI Polymer Division Citation in 1993 and the RACI Applied Research Medal in 1999. He held adjunct professorships at University of Western Australia (1992 to 2005) and at Curtin University of Technology (1999 to 2006). In 2005, he joined the newly founded Queensland Eye Institute in Brisbane, where he was offered a position of Senior Scientist to continue his research and to establish a department of ophthalmic bioengineering. Currently, he is the Chief Scientist of the institute and holds three adjunct professorships at the Queens-

land University of Technology and the University of Queensland (Australian Institute for Bioengineering and Nanotechnology, and Faculty of Health Sciences). He is a member of Australasian Society for Biomaterials and Tissue Engineering, New York Academy of Sciences, and American Chemical Society. He is on the editorial boards of 9 journals. His main research interests include polymer science, hydrogels, ophthalmic biomaterials and tissue engineering, supramolecular polymers. He is the inventor of AlphaCor™, an artificial cornea, and AlphaSphere™, an orbital implant, both in clinical. His activity has resulted to date in 160 articles and 13 patents.

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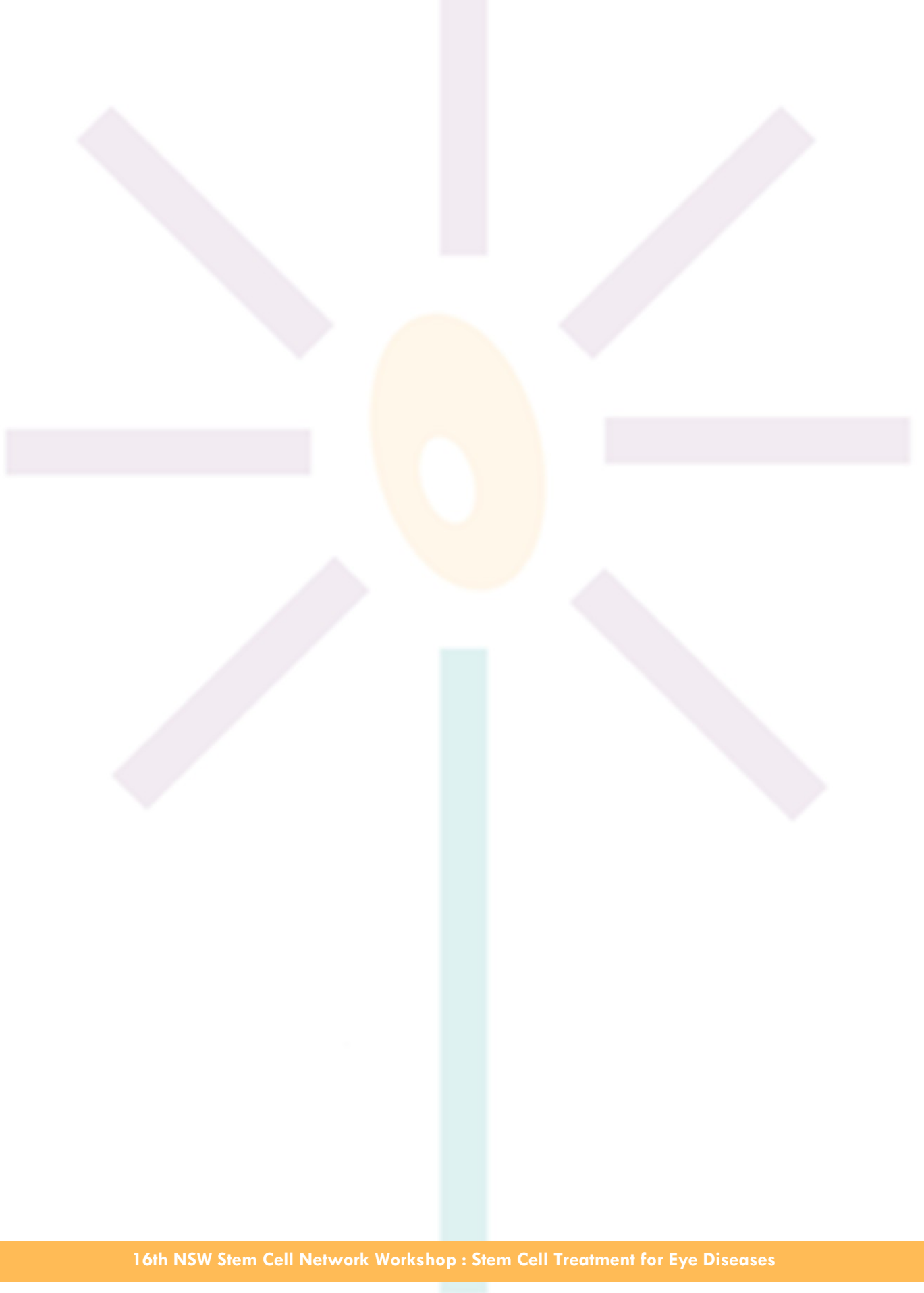
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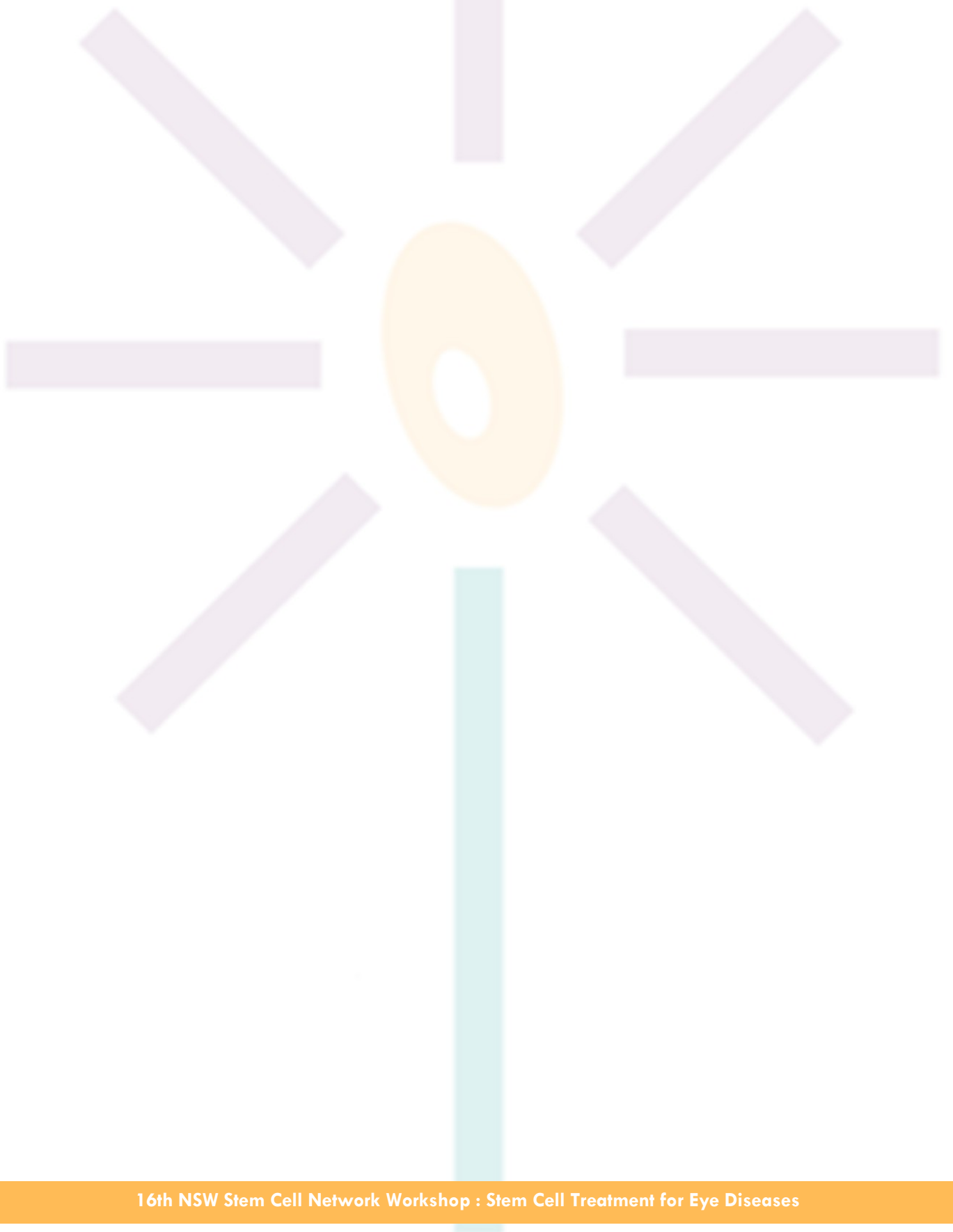
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