Review

Current status and future prospects for cultured limbal tissue transplants in Australia and New Zealand

Damien G Harkin¹,² PhD, Andrew J Apel³ FRANZCO, Nick Di Girolamo⁴ PhD, Stephanie Watson⁴,⁵ PhD FRANZCO, Karl Brown⁶,⁷, Mark D Daniell⁷ FRANZCO, Jane McGhee⁸ BSc and Charles NJ McGhee⁸ PhD FRCS.

1. School of Biomedical Sciences and Institute of Health & Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia.
2. Queensland Eye Institute, South Brisbane, Queensland, Australia.
3. Princess Alexandra Hospital, Woolloongabba, Queensland, Australia.
4. Inflammation and Infection Research Centre, School of Medical Sciences, University of New South Wales, Sydney, New South Wales, Australia.
5. Save Sight Institute, University of Sydney, Sydney, Australia.
6. O’Brien Institute, University of Victoria, Victoria, Australia.
7. Centre for Eye Research Australia, East Melbourne, Victoria, Australia.
8. Department of Ophthalmology, New Zealand National Eye Centre, Faculty of Medical and Health Sciences, University of Auckland, New Zealand.

Corresponding author:

Associate Professor Damien Harkin,
School of Biomedical Sciences, Faculty of Health, Queensland University of Technology,
2 George Street, Brisbane, Australia, 4001.
Phone: +61 7 3138 2552
Fax: +61 7 3138 6030
E-mail: d.harkin@qut.edu.au

Running title:
Cultured limbal tissue transplants

Competing or commercial interests:
Nil

Funding sources:
Nil
Abstract

Cultured limbal tissue transplants have become widely used over the last decade as a treatment for limbal stem cell deficiency (LSCD). While the number of patients afflicted with LSCD in Australia and New Zealand is considered to be relatively low, the impact of this disease on quality of life is so severe that the potential efficacy of cultured transplants has necessitated investigation. The first Australian trial of cultured limbal transplants was undertaken in Brisbane in 2002 with additional trials now having either been completed or underway in Sydney and Melbourne respectively. The first New Zealand trial of this technology commenced in Auckland in 2008 and is ongoing. Results from these studies have been encouraging and potential improvements to the technology are being actively investigated at each centre. Nevertheless, low patient numbers combined with emerging regulatory requirements for biological therapies in both countries may hamper progress from experimental status to routine clinical use. We therefore review the basic biology and experimental strategies associated with the use of cultured limbal tissue transplants in Australia and New Zealand. In doing so, we aim to encourage informed discussion between patients, clinicians, scientists, regulators and industry, on the issues required to advance the use of cultured limbal transplants in Australia and New Zealand. Moreover, we propose a business model based upon a collaborative network that could be used to maintain access to the technology in conjunction with a number of other existing and emerging biological therapies for the treatment of eye diseases.
**Introduction**

Cultured limbal tissue transplants are a specialized type of corneal tissue transplant, used for the purpose of treating limbal stem cell deficiency (LSCD)\(^1\). While relatively few patients stand to benefit from this therapy, cultured limbal transplants represent a significant milestone in the development of cellular therapies for repairing the eye. In short, the lessons learned from attempts to implement use of cultured limbal tissue transplants, will eventually be applied to the manufacture of other cultured tissues, including those for repairing the corneal stroma, corneal endothelium and the retinal pigment epithelium (RPE). These lessons relate not only to the technical aspects of growing each cell type, but also to emerging regulatory requirements for biological therapies and in particular the financial costs of their implementation\(^2\). Having pioneered the introduction of cultured limbal tissue transplants across Australia and New Zealand, we review the current status of this technology within our region with the aim to highlighting the lessons learned and challenges ahead on the road to achieving routine clinical use of cultured ocular tissue transplants. In particular, we draw attention to how the manufacture of these advanced therapies should be funded and by whom they should be produced?

**The biology of LSCD and cultured limbal transplants**

LSCD is a relatively rare disorder that can arise from a host of diseases or injuries affecting the peripheral, or limbal, margin of the cornea. Most commonly, LSCD arises from accidental exposure to corrosive chemicals or less commonly from chronic inflammation associated with immunological disorders such as Stevens-Johnson syndrome\(^1\). The developmental disorder aniridia, arising from mutations in the oculogenic PAX6 gene, is also linked to LSCD\(^3\). The anatomical significance of the limbus is that the progenitor cells required to maintain the human corneal epithelium are concentrated within this region\(^4, 5\). In the absence of a normal
limbus, there are insufficient progenitor cells to renew the corneal surface, and the cornea becomes prone to epithelial defects that result in chronic inflammation, scarring and infection. Patients afflicted with LSCD, while few in number, suffer from significant pain and vision loss, thus necessitating the search for an appropriate therapy.

Initial attempts to treat LSCD included transplants of large autologous limbal tissue segments for unilateral cases\(^6\). In bilateral cases, donor limbal transplants have been used, however, they display a high rate of rejection\(^7\), presumably owing to the presence of blood vessels and immune cells in the limbus. Autologous grafts of limbal tissue are therefore an attractive option in cases where a patient has only one eye affected by disease, however, this procedure carries the potential risk of inducing LSCD in the patient’s “donor” eye. In response to this dilemma, researchers have experimented with the idea of growing patients’ own limbal epithelial cells from a small biopsy into a sheet of tissue large enough to be applied back onto the entire corneal surface\(^8\, 9\). Autologous tissue derived from the oral or buccal mucosa has been used as a substitute for limbal tissue in the case of bilateral LSCD\(^10\).

Two basic strategies have been used to grow limbal tissue (Figure 1). The first and perhaps most widely used technique is to grow the limbal biopsy as an intact tissue explant and when sufficient growth is achieved, apply this cultured tissue back onto the ocular surface\(^11\). Alternatively, others have adopted a technique used for growing sheets of epidermal cells for burns patients whereby the biopsied cells are initially dissociated using enzymes, then grown to confluency with the aid of a surrogate “dermis” supplied by direct contact with a growth-arrested fibroblastic cell line (3T3 cells) derived from mouse embryos\(^8\, 9\). In either case, the basic aim is to produce an epithelial sheet large enough to re-surface the cornea. Each approach carries potential risks and benefits. On the one hand, serial propagation of limbal epithelial cells in the presence of 3T3 cells produces a large enough quantity of tissue to enable multiple transplants without need for further biopsies. While on the other, use of
explant cultures avoids the potential health risks associated with using mouse 3T3 cells\(^{12}\) and maintains a core culture in contact with the native extracellular matrix (stem cell niche) that may yet prove useful for retaining progenitor cell numbers \textit{in vitro}.\)

Despite use of terms such as \textquotedblleft corneal stem cell\textquotedblright{} or \textquotedblleft limbal stem cell\textquotedblright{} transplant, cultured limbal tissue consists primarily of immature corneal epithelial cells, however, efficacy has been linked to use of cultures in which at least 3\% of cells stain brightly for the progenitor cell marker \(\Delta Np63\alpha^{13}\). Donor amniotic membrane has most often been used as a substrate during limbal cell expansion\(^ {12}\) and, or transplantation, but fibrin glue\(^ {14, \ 15}\) and synthetic materials including contact lenses\(^ {16}\) have also been used successfully.

In some clinics outside Australia and New Zealand, the use of cultured limbal transplants is now considered routine and manufacturing is conducted in licensed laboratories according to local codes of good manufacturing practice (GMP)\(^ 2\). There are presently no licensed manufacturers of cultured limbal tissue transplants within Australia or New Zealand. Patients requiring access to this biological therapy may therefore only do so by seeking treatment overseas or through recruitment into a local clinical trial. To the best of our knowledge, a total of 5 clinical trials of cultured limbal transplants have either been completed or are presently underway across Australia and New Zealand. The key details of these trials are summarized in Table 1 and a case series for one of the recent trials is summarized in Table 2.

\textbf{Clinical trials in Australia and New Zealand}

\textit{Brisbane}

Two clinical trials of cultured limbal tissue transplants have been conducted in Brisbane. The first trial was led by Dr Andrew Apel between 2002 and 2003 at the Royal Brisbane &
Women’s Hospital, and the Queensland Eye Hospital. Three scientists were involved in this
study: Associate Professor Damien Harkin, a cell biologist and academic at the Queensland
University of Technology (QUT), assisted by Dr Zeke Barnard (while a PhD student), and Mr
Peter Gillies, who was at that time responsible for growing experimental sheets of skin cells
for burns patients at the Australian Red Cross Blood Service (ARCBS) in Brisbane.
Consumables costs were borne by Harkin’s research grants and labor costs were donated as
in-kind support from QUT (Harkin) and ARCBS (Gillies). Manufacturing was conducted
within a dedicated cell culture facility at ARCBS. After performing a preliminary assessment
of conditions for culturing limbal epithelial cells\textsuperscript{17}, the team adopted the skin cell culture
protocols where dissociated epithelial cells are co-cultured with 3T3 cells. The cultures were
expanded for approximately 2 weeks with the aid of 3T3 cells before sub-culturing to
confluence on donor amniotic membrane supplied by the Lions Eye Bank in Melbourne.
Excess cells were frozen in liquid nitrogen and were successfully used to generate additional
cultures for transplant on amniotic membrane. Five patients were treated during this trial, two
of which received a second transplant using cells that had been frozen following the initial
expansion. The clinical outcomes for one patient have been reported as a case study\textsuperscript{18}.
Notably, this clinical trial was terminated prematurely owing to closure of the skin cell culture
laboratory at ARCBS, thus emphasizing the importance of securing on-going access to a
dedicated manufacturing facility.

A second clinical trial was conducted in Brisbane in 2005 (CTN No. 093/2005), led by
Professor Lawrie Hirst and Professor Ivan Schwab at the Queensland Eye Institute, with Drs
Harkin and Barnard again providing a support role. This study attempted cultivation of
dissociated cells from biopsies using a commercial serum-free medium found in earlier
preliminary studies to support limbal epithelial cell growth (Defined Keratinocyte Serum-Free
Medium from Invitrogen). Moreover, an attempt was made to apply cultured cells to the
ocular surface while suspended in autologous fibrin glue\textsuperscript{14}. Significantly, no other research projects involving cell culture were being conducted at this time thus enabling the cell culture laboratory at the QEI to be dedicated to trial manufacturing. Unfortunately, this trial was abandoned after recruitment of one patient owing to poor stability of the autologous fibrin clot containing cultured cells within 24-hours following transplantation to the ocular surface. No further trials have been conducted at this facility as the laboratory is now heavily used for numerous non-clinical projects.

\textit{Sydney}

A team in Sydney has completed the world’s first trial of limbal tissue transplants cultured and transplanted while attached to contact lenses (ACTRN12607000211460)\textsuperscript{19}. The idea for this approach originated in 2006 from laboratory research conducted by Associate Professor Nick Di Girolamo (School of Medical Sciences, University of New South Wales) who demonstrated that a variety of cell types from the ocular surface could be propagated on a particular type of therapeutic contact lens\textsuperscript{19}. Following this proof-of-concept, a pilot clinical study was initiated in late 2007 using autologous cells from patients with limbal stem cell deficiency (LSCD) cultured on a contact lens in culture medium supplemented with the patient’s own serum\textsuperscript{19}. Clinical Professor Stephanie Watson conducted the trial at the Department of Ophthalmology, Prince of Wales Hospital and Sydney Eye Hospital with 3 patients. Two of the patients had LSCD following treatment for conjunctival melanoma and one patient had the genetic form of LSCD, aniridia. Cultured cells were phenotyped to ensure that progenitor cells were included in the transplanted cells. The successful results of this study were published in the journal \textit{Transplantation} in 2009\textsuperscript{16}. The advantages of this system are (i) ‘self’ cells are cultured from a small biopsy (~1 mm\textsuperscript{2}), (ii) cells are expanded from a tissue known to harbor stem cells as well as niche support factors and signals, (iii) cells are
propagated in patient’s own serum (used to promote adherence of progenitor cells) without xenogeneic components thereby reducing the risk of rejection and eliminating the need for immunosuppressive therapy, (iv) cells are not sub-cultivated, i.e. they are not exposed to enzyme solutions, hence their phenotype is less likely to change during the short culture period (10-14 days), (v) cells are cultured on an FDA-approved therapeutic contact lens which acts as a substrate, carrier, and protective shield during the attachment, growth and transfer phase, and (vi) the procedure is relatively simple, with a short treatment period, a rapid recovery phase and the potential to be repeated if necessary. The shortcomings for the procedure are (i) the location of the limbal biopsy is difficult standardize between patients, (ii) cells are cultured on an artificial surface, and (iii) the transplanted autologous cells are difficult to track, knowledge of their whereabouts would be important to inform how many transplanted cells survive and for how long. Funding to conduct a larger clinical trial in Sydney was obtained in 2010 from the Australian Stem Cell Centre. Patients with limbal stem cell failure were treated by Clinical Professor Stephanie Watson at the Sydney Eye Hospital with cells cultured in the laboratory of Associate Professor Nick Di Girolamo at UNSW. The trial was completed late in 2011 and the data is currently being analysed.

During the course of these clinical trials, Associate Professor Di Girolamo and Clinical Professor Stephanie Watson were supported by an NH&MRC Career Development Award and a Health Practitioner Training Fellowship respectively. In addition the University of NSW provided 3 years of funding towards progressing this work. The Australian Stem Cell Centre was closed in 2011 leaving a gap in potential sources of funding for stem cell researchers. The current work of this team focuses on understanding how their procedure restores the ocular surface and how it can be optimized. Animal models exist that will facilitate this work. Indeed, independent groups in Singapore and Melbourne are currently using a modification of the contact lens-based procedure in an appropriate animal model to
examine how bandage contact lenses can be used as a cell substrate and delivery device for the treatment severe ocular surface disease. 

Melbourne

A prospective interventional study of cultured limbal tissue transplants is currently underway in Melbourne. The study lacks intervention and comparison groups required to meet the International Committee of Medical Journal Editors (ICMJE) definition of a clinical trial. All patients meeting selection criteria have been offered the intervention. The study is being conducted in two phases, to assess safety and efficacy, and is led by ophthalmic surgeon Associate Professor Mark Daniell. Cultures were initially grown by Dr David Francis and Dr Trent Roydhouse (while undertaking studies towards a Masters of Surgery degree), but for the last two years cells have been grown by senior research assistant and postgraduate research student Mr Karl Brown. Dr Keren Abberton of the O’Brien Institute is also providing ongoing scientific input to this study. Cultures are being grown using an explant method, based closely on that developed at the LV Prasad Eye Institute in Hyderabad. Limbal explants have been sourced from cadaveric donor tissue, living related donors, or the patient’s contralateral eye. Use of autologous cultivated oral mucosal epithelial transplantation (COMET) as a substitute for limbal tissue has also been attempted according to established methods. Explant tissues from all sources are cultured on denuded donor amniotic membrane, originally supplied by the Lions Eye Bank in Melbourne, but currently sourced from the New Zealand National Eye Bank. The culture medium is supplemented with the patient’s own serum and is free from animal products. Using this technique, confluent sheets of limbal epithelium are being achieved within 7-14 days. During the preliminary phase of this trial to assess safety, 6 patients were treated over 5 years to 2011. Phase two is ongoing, with ethics approval to treat up to 12 patients. This work is funded by Professor Daniell’s departmental
funds, supplemented by additional funding received from the Ophthalmic Research Institute of Australia (ORIA), Australia-India Strategic Research Fund (AISRF) and the University of Melbourne.

*Auckland*

Traditional, autologous, contra-lateral sourced, limbal tissue “stem” cell transplants have been used by the ophthalmologists in the University of Auckland corneal service for nearly twenty years. However, in a manner similar to the program in Melbourne (*vide supra*), a prospective interventional study of cultured limbal tissue transplants has been underway in Auckland since 2008. Due to the relative rarity of the conditions that lead to stem cell failure, and consequent need for limbal cultured transplants, the study lacks a control arm although all patients received maximum medical and standard surgical interventions for several weeks/months prior to meeting selection criteria for inclusion (i.e. limbal stem cell failure with significant corneal epithelial compromise resistant to standard therapies).

To date 6 eyes of 5 patients have been treated by *ex-vivo* expanded epithelial cells on amniotic membrane, closely following the protocol established in Melbourne by Associate Professor Mark Daniell and Mr Karl Brown. In five subjects limbal tissue was available from the opposite eye and for one patient (patient E) no healthy limbal tissue was available, therefore a mucosal biopsy was obtained from the internal surface of the patient’s lower lip. All cultures were grown by Ms Jane McGhee with support from Associate Professor Trevor Sherwin in the Tissue Culture Suite, Department of Ophthalmology, New Zealand National Eye Centre, Faculty of Medical and Health Sciences, University of Auckland. This work was funded by departmental funds and supporting grants obtained by Professors McGhee and Sherwin.
In all patients bar one, a small biopsy (approx 1 x 1mm) of limbal tissue was taken from the healthy superior limbus of the undamaged/less damaged eye. The biopsy was placed directly onto amniotic membrane supplied and prepared by the New Zealand National Eye Bank. The amniotic membrane was stretched over an open-ended cylinder with basement membrane uppermost such that the tissue biopsy and culture medium were contained within the cylinder. After applying the biopsy, it was left for 5 minutes to settle with no predetermined orientation and then over laid with 400µl of culture medium (DMEM/F12 (Gibco) supplemented with 10% autologous serum, insulin-transferrin-selenium (Sigma), L-glutamine and antibiotics (Gibco)). After overnight culture at 37˚ and 5% CO₂, a further 1ml of medium was added. Thereafter, spent medium was replaced every three days.

Cell outgrowth from the explanted tissue was observed either by inverted microscopy and/or dark field microscopy. Spent medium was collected in order to monitor for bacterial contamination. No contamination was detected. Epithelial outgrowth and confluence was achieved in all explants (typically by day 16) with the end point (day of surgery) being no later than day 21. At this time point, cultures were transported in culture medium to the Eye Theatre at Greenlane Hospital, Auckland District Health Board for the planned intervention.

All subjects underwent superficial keratectomy and focal or complete removal of scarred limbal tissue. The 18-20 mm diameter amniotic membrane was carefully positioned with the cell culture innermost and the membrane was secured with 12 peripheral sutures of 10/0 Vicryl. In patients A and B, a penetrating keratoplasty (PKP) was performed at a later date when the ocular surface had stabilized. In case A* this was associated with a second limbal cell transplant to accompany the corneal transplant (Figure 2). In patient C, a simultaneous PKP was performed in a subject whose main indication was relief of pain in an eye with a highly unstable ocular surface. All limbal cell transplants were successful in terms of producing a stable, comfortable ocular surface and clear cornea (Table 2) with variable
improvement of visual acuity. Unfortunately, the single case of expanded buccal mucosa in an extremely dry, Stevens Johnson Syndrome eye, failed within 2 months.

**Future access to treatment in Australia and New Zealand**

Once any new therapy has been determined safe and beneficial to patients, it will generally become available on a more routine basis. Cultured limbal tissue transplants have been shown to be safe and while there remains room for improvement, the bulk of clinical data from both within and outside the region indicates that these treatments reduce pain, improve cosmesis and can partially restore vision\(^1\)\(^,\)\(^{13,22,23}\). Notably, best overall results are often obtained when used in conjunction with conventional surgical treatments such as penetrating keratoplasty (as shown previously by Harkin et al. (2004)\(^\text{18}\) and presently in Table 2 and Figure 2). Thus it seems logical that this technology should in time advance beyond its experimental status in Australia and New Zealand, however, there are a number of issues to be resolved before achieving this goal.

A number of hurdles need to be overcome to progress the use of cultured limbal tissue transplants in Australia and New Zealand. These include resources, time and effort required to establish and maintain a licensed manufacturing facility\(^2\). According to the recently updated regulatory framework for biological therapies developed by the Therapeutic Goods Administration of Australia (TGA), cultured limbal tissue transplants seem likely to be classified as a category 3 biological (owing to *ex vivo* expansion of cells and potential use of digestive enzymes that may alter their properties). Steps required in the licensing of category 3 biologicals include preparation of a dossier outlining in detail all processes used during manufacture ranging from sourcing of materials used, up until methods of final product storage and delivery back to the patient. Preparation of the dossier is anticipated to be a particularly time-consuming process. The dossier is submitted to TGA for evaluation to
ensure compliance with default manufacturing standards. Moreover, the facility in which the biological is produced must hold a current TGA manufacturing license that is issued after demonstration of compliance with manufacturing principles including those outlined in the *Australian Code of GMP for Human Blood and Tissues*. The fees associated with these regulatory requirements are summarized in Table 3. In order to alleviate these costs, the Australian government has committed funds during the first three years of the new regulatory framework (31st May 2011 until 31st May 2014) to cover the direct regulatory costs associated with implementation, but only in the case of publicly funded organizations and not-for-profit hospital supply units. Thus there is a clear incentive for organizations that fall within this category to galvanize their efforts during this window of opportunity. The regulatory landscape in New Zealand seems destined to follow a similar path given the merger between Australian (Therapeutic Goods Administration) and New Zealand (Medsafe) regulatory authorities currently in progress.

Assuming that the initial regulatory fees can be waived, there remain additional costs associated with establishing and maintaining the required laboratory infrastructure, as well as paying staff salaries. In particular, a salary would need to be provided to support the application process to the regulator. Closely coupled with these considerations is the fact that patients with LSCD are considered less common in Australia and New Zealand, thus making it potentially unfeasible from an economic perspective to establish and maintain a laboratory solely for the purpose of treating this disease. By comparison, Mesoblast Ltd (http://www.mesoblast.com/), a company that specializes in the manufacture of cultured bone marrow mesenchymal stromal cells, is marketing this biological therapy for a range of common health conditions including musculoskeletal defects and cardiovascular disease. Significant lessons can also be gained by considering the history of cultured skin tissue transplants in Australia and New Zealand, which after several years of manufacture by not-
for-profit organizations and hospital supply units, are now required to be produced within licensed facilities\textsuperscript{24, 25}.

In considering the above, we propose that a dedicated not-for-profit facility should be established to serve the needs of LSCD patients in Australia, New Zealand as well as patients from other countries within the region. This facility could also be responsible for manufacturing processed donor amniotic membrane, which in addition to being used as a therapeutic bandage for the treatment of corneal diseases, could potentially be incorporated into standardized protocols for culturing limbal tissue. Looking further ahead, the techniques that are currently being developed in Australia and New Zealand for the \textit{ex vivo} expansion of corneal endothelial cells\textsuperscript{26} and limbal stromal cells\textsuperscript{27} could also be incorporated into the list of manufactured products thus greatly increasing the number of patients who would receive benefit from this facility. Alternatively, given the costs and logistics of patient transport, it may well prove more feasible to maintain laboratories in multiple centres, with a reduction in manufacturing costs achieved through adoption of standardized protocols throughout Australia and New Zealand. In doing so, the technology could be administered through a similar collaborative network as currently exists for the supply of donor eye tissue in Australia and New Zealand\textsuperscript{7}. As such, we would further propose that the Eye Bank Association of Australia and New Zealand (EBAANZ), that represents the interests of eye banks in these countries, could play a vital role in facilitating initial discussions across state and national borders on this issue.

\textbf{Conclusions}

Cultured limbal tissue transplants have proven to be an effective step forward in the treatment of LSCD. Several clinics within Australia and New Zealand have developed the expertise required to cultivate limbal tissue for transplantation under the auspices of clinical trials.
Translation of this therapy from experimental status to routine use, however, will require manufacture within a GMP-licensed facility. The economic logistics of this endeavor will most likely necessitate a collaborative approach within the region and perhaps can be facilitated by combining with other orphaned and emerging therapies for ocular diseases. In the short term, we propose that the existing network of eye tissue banks within the region (EBANZ), together with the Royal Australian and New Zealand College of Ophthalmologists (RANZCO) and affiliated research scientists, should establish a standardized protocol for the manufacture of cultured limbal tissue transplants in Australia and New Zealand.

Acknowledgements

The authors acknowledge the assistance of the numerous patients, clinicians and scientists associated with the trials reviewed in this paper. In particular, we wish to acknowledge Professor Ivan Schwab (University of California at Davis, USA) and Professor Lawrie Hirst (Queensland Eye Institute, Brisbane, Australia) for reviewing the description of the second clinical trial in Brisbane, and Louise Moffatt (New Zealand National Eye Bank, University of Auckland) for reviewing comments on the pending regulatory changes in New Zealand. We also thank Mr Thomas Hogerheyde (Queensland University of Technology, Brisbane, Australia) for his assistance in obtaining the image shown in Fig 1B. While no funding was received to support preparation of this review paper, the sources of funding relevant to each clinical trial are summarized in Table 1. None of the authors have a financial interest in any of the products or procedures described in this paper.
References


Figures

Figure 1. Demonstration of the two basic strategies used for growing cultured limbal tissue transplants. (A) *Explant technique*: a small biopsy of patient limbal tissue is grown intact as an explant while attached to some form of support substrate. The image displays epithelial cell outgrowth beyond the edge of the explant (dark centre) after two days culture on a Lotrafilcon A™ contact lens in culture medium supplemented with 10% (v/v) patient serum. The patient received the culture after approximately 10 days, at which time the entire surface of the contact lens was covered with epithelial cells. (B) *Ex-vivo expansion in presence of murine 3T3 cells*: a similar sized biopsy to the one shown in part A is digested using enzymes to create a cell suspension then seeded into culture dishes containing growth-arrested murine 3T3 cells. The image displays an island of limbal epithelial cells (surrounded by 3T3 cells) that is typical of that observed within the first 7 days of culture. Using this approach, approximately 10 million cells are available for transplantation within 2 weeks (sufficient for at least 5 treatments).
Figure 2. Example of clinical outcome for patient treated with cultured limbal tissue transplant (Auckland study Patient A*: use in conjunction with penetrating keratoplasty). (A) Appearance of eye demonstrating extensive scarring and vascularisation of cornea following an alkaline chemical injury (BCVA = “counting fingers” only. (B) Appearance of eye immediately following performing penetrating keratoplasty (PKP) in conjunction with an overlying graft of cultured limbal tissue grown on amniotic membrane. (C) Appearance of same eye 12 months following combined grafting procedure (BCVA = 6/12).
**Table 1.** Summary of clinical trials in Australia and New Zealand examining the use of cultured limbal tissue transplants.

<table>
<thead>
<tr>
<th>Study</th>
<th>Manufacturing facility</th>
<th>Funding sources</th>
<th>Culture method</th>
<th>Source of tissue</th>
<th>Substrate</th>
<th>Trial status</th>
<th>No. of patients</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apel &amp; Harkin 2002-2003</td>
<td>ARCBS¹ in Brisbane</td>
<td>QUT², ARCBS, Royal Brisbane and Women’s Hospital Research Foundation</td>
<td>Dissociated co-culture with murine 3T3 cells.</td>
<td>Autologous</td>
<td>Amniotic membrane</td>
<td>Complete</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Hirst, Schwab &amp; Harkin 2006</td>
<td>Queensland Eye Institute</td>
<td>Research to Prevent Blindness, UC Davis, Queensland Eye Institute, QUT</td>
<td>Dissociated culture grown in commercial serum-free medium</td>
<td>Autologous</td>
<td>Autologous fibrin glue (Cryoseal)</td>
<td>Complete</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Watson &amp; Di Girolamo 1. 2007 2. 2010-2011</td>
<td>School of Medical Sciences UNSW³</td>
<td>1. School of Medical Sciences, UNSW 2. Australian Stem Cell Centre</td>
<td>Explant cultures grown on contact lenses</td>
<td>Autologous</td>
<td>Contact lens</td>
<td>Complete</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Daniell, Abberton &amp; Brown 2007-present</td>
<td>Centre for Eye Research Australia and the O’Brien Institute</td>
<td>University of Melbourne, ORIA⁴ &amp; AISRF⁵.</td>
<td>Explant cultures grown on amniotic membrane</td>
<td>Autologous/allogeic limbus and autologous oral mucosa</td>
<td>Amniotic membrane</td>
<td>In progress</td>
<td>6+</td>
<td>-</td>
</tr>
<tr>
<td>McGhee, Sherwin &amp; McGhee 2008-present</td>
<td>Department of Ophthalmology University of Auckland</td>
<td>Department of Ophthalmology University of Auckland</td>
<td>Explant cultures grown on amniotic membrane</td>
<td>Autologous limbus or oral mucosa</td>
<td>Amniotic membrane</td>
<td>In progress</td>
<td>6+ (refer to Table 2)</td>
<td>-</td>
</tr>
</tbody>
</table>

¹Australian Red Cross Blood Service. ²Queensland University of Technology. ³University of New South Wales. ⁴Ophthalmic Research Institute of Australia. ⁵Australia-India Strategic Research Fund.
Table 2: Case series of cultured tissue transplants for the treatment of LSCD (Auckland study). Details of six eyes of five subjects (A to E) with M:F ratio 4:1, treated for limbal stem cell deficiency by autologous, *ex vivo* expanded, limbal cell transplants on human amniotic membrane (except case E which used expanded buccal mucosa). Pre-operative best-corrected visual acuity (BCVA) was between 6/24 to “Perception of Light” (P of L) and the majority of eyes achieved improved vision following surgery. One eye (patient E) had limited visual potential due to retinal disease. Four of five patients also underwent penetrating keratoplasty (PKP) being time separated in two cases (A* and B). One case had two stem cell expansions on amniotic membrane (A and A*). Four of five eyes maintained restoration and stability of the ocular surface at 9-36 months. (CF = counting fingers vision).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender /Age</th>
<th>Cause of LSCD</th>
<th>Indication</th>
<th>Pre-op BCVA</th>
<th>Post-op BCVA</th>
<th>General outcome</th>
<th>Follow-up (Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M /37</td>
<td>Alkali injury</td>
<td>Ocular surface restoration</td>
<td>CF</td>
<td>CF</td>
<td>Stable corneal surface</td>
<td>6</td>
</tr>
<tr>
<td>A*</td>
<td>M / 38</td>
<td>Alkali injury</td>
<td>Ocular surface restoration plus PKP</td>
<td>CF</td>
<td>6/12</td>
<td>Clear cornea (refer to Figure 2).</td>
<td>30</td>
</tr>
<tr>
<td>B</td>
<td>M / 23</td>
<td>Firework: chemical &amp; thermal</td>
<td>Ocular surface restoration plus PKP</td>
<td>6/36</td>
<td>6/9</td>
<td>Clear cornea</td>
<td>24</td>
</tr>
<tr>
<td>C</td>
<td>F / 46</td>
<td>PKP/RD &amp; multiple operations</td>
<td>Ocular surface restoration plus PKP</td>
<td>CF</td>
<td>6/36</td>
<td>Clear cornea</td>
<td>12</td>
</tr>
<tr>
<td>D</td>
<td>M / 42</td>
<td>Thermal injury boiling water</td>
<td>Ocular surface restoration</td>
<td>6/24</td>
<td>6/18</td>
<td>Clear cornea</td>
<td>9</td>
</tr>
<tr>
<td>E</td>
<td>M / 63</td>
<td>Steven-Johnson syndrome</td>
<td>Ocular surface restoration plus PKP NB: buccal mucosal tissue used in absence of available limbal tissue.</td>
<td>P of L</td>
<td>P of L</td>
<td>Failed</td>
<td>9</td>
</tr>
</tbody>
</table>
Table 3. Summary of regulatory fees associated with applying for and maintaining a TGA-licensed facility to manufacture a category 3 biological such as cultured limbal tissue transplants (adapted from http://www.tga.gov.au/about/fees.htm). Additional application and evaluation fees are associated for minor and major amendments to the manufacturing protocol. Not-for-profit organizations and hospital supply units have until to 31st May 2014 to apply for a fee exemption. A similar pricing structure seems likely to be adopted in NZ in view of current merger of regulatory authorities to create the Australia New Zealand Therapeutic Products Agency (ANZTPA).

<table>
<thead>
<tr>
<th>Description</th>
<th>Fee¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation of dossier for a Class 3 biological</td>
<td>$120,000</td>
</tr>
<tr>
<td>Application for manufacturing license</td>
<td>$900</td>
</tr>
<tr>
<td>Application for Inclusion of a class 1, 2, 3, 4 biological in the ARTG²</td>
<td>$900</td>
</tr>
<tr>
<td>Domestic initial manufacturing audit</td>
<td>$17,990</td>
</tr>
<tr>
<td>Annual charge for inclusion of a Class 2,3 or 4 biological in the ARTG</td>
<td>$5,550</td>
</tr>
<tr>
<td>Domestic subsequent manufacturing audit</td>
<td>$13,500</td>
</tr>
</tbody>
</table>

¹ Australian dollars. ² Australian Register of Therapeutic Goods.