PROSPECT
Journal of Cellular Biochemistry 112:993–1002 (2011)

Journal of Cellular Biochemistry

13 Years of Cultured Limbal Epithelial Cell Therapy: A Review of the Outcomes

Oliver Baylis,[1,2,3] Francisco Figueiredo,[1,2,3] Christin Henein,[1,2] Majlinda Lako,[1,2] and Sajjad Ahmad[1,2,3]*

[1]*Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, UK*
[2]*North East England Stem Cell Institute, Newcastle University, Newcastle upon Tyne, UK*
[3]*Department of Ophthalmology, Royal Victoria Infirmary, Newcastle University, Newcastle upon Tyne, UK*

ABSTRACT

The cornea is the clear tissue at the front of the eye which enables the transmission of light to the retina for normal vision. The surface of the cornea is composed of an epithelium which is renewed by stem cells located at the periphery of the cornea, a region known as the limbus. These limbal stem cells can become deficient as a result of various diseases of the eye's surface, resulting in the blinding disease of limbal stem cell deficiency. The treatment of this disease is often difficult and complex. In 1997, it was proposed that a small amount of limbal tissue containing limbal stem cells could be culture expanded and then transplanted. Since then various case reports and case series have been reported showing promising results. Here, we review the outcomes of this procedure over the past 13 years with the aim of highlighting the best culture and surgical techniques to date. J. Cell. Biochem. 112: 993–1002, 2011. © 2011 Wiley-Liss, Inc.

KEY WORDS: CORNEA; LIMBUS; LIMBAL STEM CELL; LIMBAL STEM CELL DEFICIENCY; CULTURE; TRANSPLANT

The cornea is the clear window at the front of the eye. Its clarity enables the transmission of light to the retina for normal visual perception. The corneal surface is made up of an epithelium which is renewed by stem cells located at the edge of the cornea, in a region known as the limbus. With regard to the corneal epithelium, the limbus has two important roles: firstly it harbors stem cells for the corneal epithelium, and secondly it acts as a barrier preventing the hazier conjunctival epithelium and its blood vessels, which surround the limbus, from encroaching on to the corneal surface (Fig. 1). When disease or injury results in limbal and limbal stem cell damage, these two roles of the limbus fail. The painful and blinding disease of limbal stem cell deficiency then ensues [Ahmad et al., 2006].

Total and severe limbal stem cell deficiency is a difficult and complex disease to manage. The management options vary from symptom control using conservative measures such as bandage contact lenses to more definitive treatment with surgery. It must be noted that corneal transplantation which involves replacing the central cornea (excluding the limbus) cannot be used as a treatment option for limbal stem cell deficiency due to the lack of host limbal stem cells to replace the epithelium overlying the corneal graft [Ahmad et al., 2010a,b]. In 1989, it was first proposed that limbal stem cell deficiency could be successfully treated using limbal tissue grafts [Kenyon and Tseng, 1989]. This procedure has significant disadvantages which often prevent patients from undergoing it. These disadvantages include the large amounts of limbal tissue needed which risks inducing limbal stem cell deficiency to the donor eye, and in cases of allogeneic tissue, the requirement of potent immune suppression which poses the risks of life-threatening opportunistic infections and neoplasia.

In 1997, it was first proposed that much smaller pieces of limbal epithelium could be removed from the healthy donor eye, expanded in culture, and then transplanted to the recipient eye following removal of the conjunctival tissue from the corneal surface [Pellegrini et al., 1997]. Over the past 13 years there have been various modifications of the culture and transplantation techniques. In the case series and reports published, the results seem to be promising. However, due to variations within the studies and between studies, it is often difficult to make an objective assessment. Culture techniques and outcomes were reviewed by Shortt et al. [2007]. Since then there has been further publication of results and this article aims at reviewing all published case series and reports over the past 13 years in order to assess the outcomes of the culture and transplantation of human limbal epithelium.

*Correspondence to: Dr Sajjad Ahmad, Institute of Human Genetics, Newcastle University, Central Parkway, Newcastle upon Tyne NE1 3BZ, UK. E-mail: sajjad.ahmad@ncl.ac.uk
Received 6 January 2011; Accepted 11 January 2011 • DOI 10.1002/jcb.23028 • © 2011 Wiley-Liss, Inc.
Published online 1 February 2011 in Wiley Online Library (wileyonlinelibrary.com).

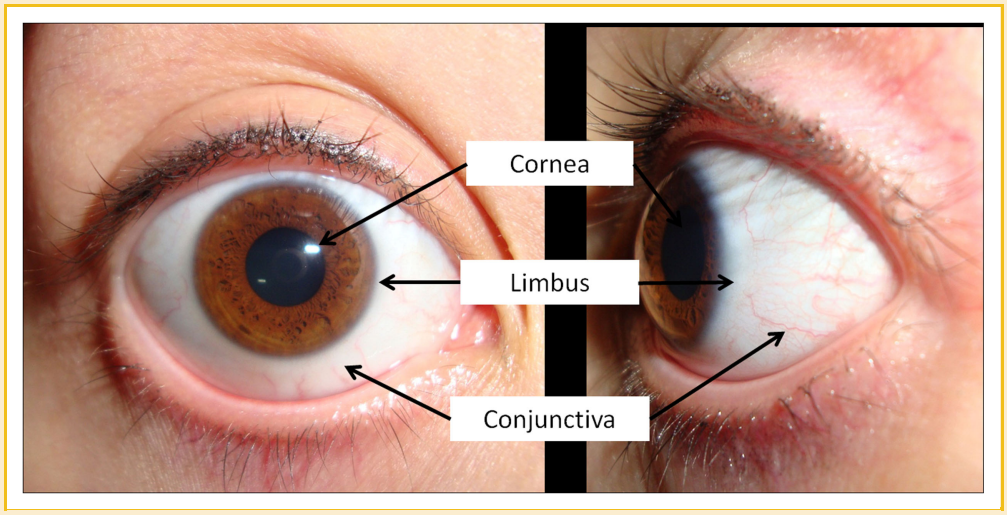

Fig. 1. Photograph of a normal human eye showing the normal appearance and the location of the cornea, limbus, and conjunctiva.

GENERAL ANALYSIS OF THE STUDIES

This review includes 28 case reports and series published over the past 13 years [Pellegrini et al., 1997; Schwab, 1999; Schwab et al., 2000; Tsai et al., 2000; Rama et al., 2001, 2010; Koizumi et al., 2001a,b; Grueterich et al., 2002; Nakamura et al., 2003, 2004, 2006; Sangwan et al., 2003, 2005, 2006; Daya et al., 2005; Ang et al., 2007; Fatima et al., 2007; Kawashima et al., 2007; Shimazaki et al., 2007; Shortt et al., 2008; Di Girolamo et al., 2009; Meller et al., 2009; Baradaran-Rafii et al., 2010; Colabelli Gisoldi et al., 2010; Di Iorio et al., 2010; Kolli et al., 2010; Pauklin et al., 2010]. There were 10 studies with 5 patients or less, 13 studies with 6–20 patients, and 5 with more than 20 patients. The centers undertaking cultured limbal epithelial transplantation are spread across the world, including Australia, Germany, India, Iran, Italy, Japan, Taiwan, UK, and USA. In total, data from 583 patients (597 eyes) was included in this review. The age range of the patients was between 3 and 91 years (mean 42 years). The male to female ratio was approximately 3:1.

ETIOLOGY OF LIMBAL STEM CELL DEFICIENCY

The causes of limbal stem cell deficiency include hereditary and acquired causes. In the studies reviewed, chemical or thermal trauma was by far the commonest cause in 449 eyes (75%) cases. Inflammatory eye disease, for example resulting from Stevens–Johnson syndrome and ocular cicatricial pemphigoid, resulted in 7.8% cases (47 eyes). Hereditary causes, such as Aniridia and ectodermal dysplasia, resulted in 2.5% of cases (15 eyes). Other causes account for the remaining 14% of cases (86 eyes) and include recurrent pterygia and iatrogenic causes such as limbal surgery, Mitomycin C treatment, and radiation therapy. On the whole, the majority of patients are young males, treated for burns, which may reflect their greater risk.

DIAGNOSTIC CRITERIA FOR PATIENTS WITH LIMBAL STEM CELL DEFICIENCY

CLINICAL ASSESSMENT

The most prominent feature of limbal stem cell deficiency is conjunctivalization of the cornea, and signs include corneal vascularization, corneal epithelial defects, scarring, and late staining with fluorescein [Dua and Azuara-Blanco, 2000]. The details of clinical assessment vary between studies. Some simply state the diagnosis on clinical grounds whereas others attempt to make this a more objective assessment with scoring systems. It must be noted that case studies with few patients are able to include detailed clinical descriptions not possible with larger numbers. Limbal stem cell deficiency was total in 461 eyes overall (77%), and partial in 136 (23%). However, there is inconsistency and different studies defined these terms differently, whether purely a clinical diagnosis or on the basis of corneal impression cytology scoring (see below). Photographs were included in nearly all articles as illustrations, but only a few gave details of photography as an outcome measure as part of a trial protocol.

ASSESSMENT OF ADNEXA AND DRY EYES

The ocular adnexa are the structures around the eye that are important for maintaining the health of the eye and the ocular surface. These include the lids, eyelashes, conjunctiva, and lacrimal system. Adnexal assessment, including dry eye assessment is important as any adnexal disease can compromise the outcomes of any surgery for limbal stem cell deficiency. It is therefore vital to resolve all adnexal diseases prior to consideration of limbal surgery. Details of any adnexal examination were on the whole poorly recorded if at all. The assessment of dry eyes was specifically detailed in 9 studies and with Schirmer's test in 6. Daya et al. [2005] performed punctal occlusion in all cases prior to limbal stem cell transplantation to aid ocular lubrication. Three studies described complications of surgery related to exposure or lid deformities

that had not been corrected preoperatively [Baradaran-Rafii et al., 2010; Koizumi et al., 2001a; Pauklin et al., 2010]. Meller et al. [2009] did not perform cultured limbal epithelial transplantation initially due to dry eye in one patient but it was performed later as this parameter improved through systemic therapy. DeSousa et al. [2009] describe a series of 29 patients who had limbal stem cell transplants by different techniques. They looked at stem cell transplant failure rates and how these related to adnexal problems. Ten patients (34%) had previous adnexal surgery and 19 (66%) had adnexal abnormalities in the period after cultured limbal epithelial transplantation that required surgery. The stem cell transplant failure rate was 58% for those who had prior forniceal abnormalities compared to 12% in those who did not. For those who required subsequent adnexal surgery the stem cell failure rate was 37% compared to 20% who did not. The authors suggested that adnexal abnormalities were not always diagnosed until problems occurred such as persistent epithelial defects. Therefore, they recommended systematic examination of lid position, lacrimal drainage, and fornix depth, followed by surgical correction prior to any surgical treatment of the limbal stem cell deficiency [DeSousa et al., 2009].

CORNEAL IMPRESSION CYTOLOGY

Corneal impression cytology of the corneal surface may be used to provide cytological evidence of limbal stem cell deficiency. A nitrocellulose filter paper is gently pressed against the cornea under topical anesthesia to remove the most superficial cells. These cells are then examined to look for evidence of conjunctival epithelium on the cornea. The first method used was to search for goblet cells, which characteristically appear within conjunctival but not corneal epithelium [Puangsricharern and Tseng, 1995]. However, this technique lacks sensitivity compared to immunocytochemical techniques that look specifically at cytokeratin markers expressed by differentiated epithelial cells. In particular cytokeratins 3 and 12 are present in normal corneal epithelial cells, whereas cytokeratin 19 is expressed by conjunctival epithelium. The cytokeratin profile has been shown to correlate well with the clinical findings and can confirm or refute the diagnosis of limbal stem cell deficiency [Sacchetti et al., 2005]. One important limitation of corneal impression cytology however is that if an inadequate number of cells is removed, the results may be unreliable.

Impression cytology was used in 11 studies to confirm the diagnosis of limbal stem cell deficiency. Specifically, histological stains were documented as being used in 3 studiess and immunocytochemical techniques in 4 studies. Cytokeratins 3 and 19 staining was used to identify the distribution of corneal and conjunctival epithelial cells. This can also be expressed as a ratio to define the severity of limbal stem cell deficiency. Of note, Rama et al. [2010] actually stopped performing impression cytology early in their study as they reported significant post-procedure pain due to epithelial defects. Also, they felt that it added little to the clinical assessment to justify it. This is potentially a problem especially as patients with limbal stem cell deficiency do have an unstable corneal surface. However, at present it remains the most practical way to confirm the diagnosis of LSCD, especially in cases of clinical doubt. Also this test is very important to perform in the fellow eye of presumed unilateral cases, to look for subclinical limbal stem cell deficiency which could either be made manifest by limbal tissue removal or predict culture failure. As shown by Rama cultures that contain inadequate numbers of stem cells are associated with poor clinical results following transplantation [Rama et al., 2010].

CONFOCAL MICROSCOPY

In vivo confocal microscopy is a technique in which the light source and the condensing lens of the microscope are focused on the same point. Live tissue is viewed parallel rather than perpendicular to its surface and thus it has high resolution with minimal interference from more superficial and deeper layers. Although most confocal microscopes require a contact technique, no actual tissue is removed. Individual cells can be imaged and the different histological layers and cell types identified. Normal corneal epithelial cells appear well defined and regular, with bright borders and dark cytoplasm. In the superficial layers, they are flatter and have bright nuclei. They can be clearly differentiated from conjunctival epithelial cells which are hyper-reflective and ill-defined [Dua et al., 2009]. In addition, conjunctival tissue contains goblet cells and blood vessels which can be seen using this technique. Thus, confocal microscopy can assist in establishing the clinical diagnosis of limbal stem cell deficiency. It can also be used to assess outcomes of surgery (i.e., restoration of normal corneal epithelium) at a cellular level without removing tissue. Confocal microscopy was only used in one study in this series, but it shows great promise as an in vivo assessment of corneal cells [Shortt et al., 2008]. Shortt et al. took confocal pictures of the epithelial layer and also the layer 5 μm above Bowman's layer using the Rostock Corneal Module and Heidelberg HRT-II (Gmbh, Germany). The pictures were then analyzed and classed as either a corneal or conjunctival phenotype.

PREVIOUS TREATMENTS AND OPERATIONS

A number of patients had had previous surgery for their limbal stem cell deficiency, including corneal transplantation (124 eyes), amniotic membrane transplantation (92 eyes), and previous limbal tissue transplantation (37 eyes). More than a third of patients had therefore had previous, presumably failed, surgery for their limbal stem cell deficiency. As patients without and with previous surgery both have been included within the data in the studies, it must be noted that it is difficult to interpret whether previous failed surgery has an impact on the outcome of cultured limbal epithelial transplantation.

CULTURE METHODS

SOURCE OF DONOR LIMBAL TISSUE

Allogeneic limbal tissue was used for culture in 5 studies, autologous material was used in 14, and both were used in 9 studies (Table I). In total, allogeneic tissue from a cadaveric source was used for 69 transplants, living related donor material was used

TABLE I. Culture Methods

Refs.	Allogeneic/ autologous	Explant/ suspension	Substrate	3T3s used	Nutrient	Air-lifting	Animal free	GMP	Culture time (days)
Ang et al. [2007]	Allogeneic (cadaveric)	Suspension	HAM (denuded)	Yes	FCS	Yes	No	No	21
Baradaran-Rafii et al. [2010]	Autologous	Explant (with dispase)	HAM (denuded)	No	FCS	No	No	No	14
Colabelli Gisoldi et al. [2010]	Autologous	Suspension	Fibrin	Yes	FCS	No	No	Yes	14–16
Daya et al. [2005]	Allogeneic (1 LR, 9 cadaveric)	Suspension	3T3s	Yes	FCS	No	No	No	12
Di Girolamo et al. [2009]	Autologous	Explant	Contact lens	No	AS	No	Yes	No	10
Di Iorio et al. [2010]	Autologous	Suspension	Fibrin	Yes	FCS	No	No	No	x
Fatima et al. [2007]	Autologous	Explant (shredded)	HAM	No	FCS	No	No	No	10–15
Grueterich et al. [2002]	Autologous	Explant	HAM	No	FCS	No	No	No	21
Kawashima et al. [2007]	2 autologous 4 allogenic (1 LR, 3 cadaveric)	Explant	HAM (denuded)	Yes	FCS or AS	Yes	No	No	x
Koizumi et al. [2001a]	Allogeneic (cadaveric)	Explant	HAM (denuded)	Yes	FCS	Yes	No	No	28
Koizumi et al. [2001b]	Allogeneic (cadaveric)	Explant	HAM (denuded)	Yes	FCS	Yes	No	No	28
Kolli et al. [2010]	Autologous	Explant	HAM	No	AS	No	Yes	Yes	12–14
Meller et al. [2009]	Allogeneic[a]	Explant	HAM	No	AS	No	Yes	No	x
Nakamura et al. [2003]	Allogeneic (cadaveric)	Explant	HAM (denuded)	Yes	FCS	Yes	No	No	28
Nakamura et al. [2004]	Autologous	Explant	HAM (denuded)	Yes	FCS	Yes	No	No	23
Nakamura et al. [2006] [b]	Autologous	Explant	HAM (denuded)	No	AS	Yes	Yes	No	15–16
	Allogeneic (cadaveric)	Suspension	HAM (denuded)	Yes	FCS	Yes	No	No	15–16
Pauklin et al. [2010]	30 autologous 4 allogenic (4 LR, 10 cadaveric)	Explant (with dispase)	HAM	No	AS	No	No	No	14
Pellegrini et al. [1997]	Autologous	Suspension	3T3s	Yes	FCS	No	No	No	16–19
Rama et al. [2001]	Autologous	Suspension	Fibrin	Yes	FCS	No	No	No	14–16
Rama et al. [2010]	Autologous	Suspension	Fibrin	Yes	FCS	No	No	Yes	14–16
Sangwan et al. [2003]	Autologous	Explant (shredded)	HAM	No	FCS	No	No	No	11–15
Sangwan et al. [2005]	11 autologous 4 allogenic (3 LR, 1 unrelated)	Explant (shredded)	HAM	No	FCS	No	No	No	10–15
Sangwan et al. [2006]	Autologous	Explant (shredded)	HAM	No	FCS	No	No	No	10–14
Schwab 1999	17 Autologous 2 allogenic (2 LR)	Suspension	HAM	Yes	FCS	No	No	No	28–35
Schwab et al. [2000]	10 Autologous 4 allogenic (4 LR)	Suspension	HAM (denuded)	Yes	FCS	Yes	No	No	21–28
Shimazaki et al. [2007] [b]	2 Autologous 14 allogenic (7 LR, 7 cadaveric)	Explant (cut up)	HAM (denuded)	No	AS	No	Yes	No	14.6
	5 Autologous 6 allogenic (1 LR, 5 cadaveric)	Suspension	HAM (denuded	Yes	AS	Yes	No	No	20.8
Shortt et al. [2008]	3 Autologous 7 allogenic (7 cadaveric)	Suspension	HAM (denuded)	No	FCS	No	No	Yes	14–21
Tsai et al. [2000]	Autologous	Explant	HAM	No	FCS	No	No	No	14–21

Different elements of culture techniques are shown. The cultured limbal tissue may be autologous or allogeneic.
LR, living related donor; HAM, human amniotic membrane; CL, contact lens; FCS, fetal calf serum; AS, autologous serum; GMP, good medical practice; 3T3, 3T3 mouse fibroblasts.
[a]HLA donor—also donated blood for blood stem cell transplant.
[b]These two studies used two different techniques.

in 23 and 1 patient received tissue from an unrelated living donor. Allogeneic transplantation therefore accounted for 16% of the cases. The other 504 transplants (84%) were autologous. Normally the autologous limbal tissue was taken from the fellow eye, but in some cases it was taken from a healthy area of the affected eye when there was partial disease (even in bilateral cases) [Sangwan et al., 2003].

SUSPENSION VERSUS EXPLANT CULTURE

Culture conditions for human limbal epithelial cells have recently been reviewed [Osei-Bempong et al., 2009]. The main differences between studies were whether the explant or cell suspension method was used, the usage or not of 3T3 mouse fibroblasts to co-culture, the type of substrate, and the culture medium used (Table I). In addition, some groups employed airlifting to produce stratified epithelium. Some groups used animal-free culture and/or good manufacturing practice conditions. The suspension method of cultures was used in 12 studies and the explant one in 18, with 2 of the studies employing both methods [Nakamura et al., 2006; Shimazaki et al., 2007].

CULTURE COMPOSITION: 3T3 FIBROBLASTS, AMNIOTIC MEMBRANE, AND FIBRIN

Cultures were grown using 3T3 cells during the culture process in 16 studies. 3T3 cells are mouse fibroblast cells that have been used in tissue culture for many years to allow epithelial cells to form uniform layers. However, there is a theoretical risk of transplanting xenogenic tissue, in terms of infection, rejection, or microchimerism [Schwab et al., 2006]. In order to minimize potential risks, some

groups use only clinical grade tissue, and some only use 3T3 cells at the beginning of the culture before plating onto a secondary substrate [Schwab et al., 2000; Rama et al., 2010].

Human amniotic membrane is non-immunogenic and has been used in medical practice for 100 years for wound healing and surgical applications [Dua et al., 2004]. Human amniotic membrane was used as a culture substrate in the majority of studies (21 studies), and the amniotic membrane was de-epithelialized or denuded prior to culture in 11 of these studies (Table I). Fibrin was used as a substrate in only 4 studies, although due to the numbers of patients in these studies, this accounted for 303 transplants, more than half of the total. Two studies (12 patients) had cells grown on 3T3 cells alone [Pellegrini et al., 1997; Daya et al., 2005]. In one study, the limbal epithelial cells were grown directly onto a contact lens without the need for 3T3 cells and the cells were transplanted simply by inserting the contact lens [Di Girolamo et al., 2009].

SERUM IN CULTURE MEDIUM

Fetal calf serum has been used extensively in epithelial culture systems. However, in recent years the risk of acquiring prion diseases has meant that alternatives to bovine and other animal products must be found wherever possible [Schwab et al., 2006]. Autologous human serum can be used as an alternative to fetal calf serum in the culture of limbal epithelial cells [Nakamura et al., 2006]. Autologous serum is made from the donated blood of the same patient. Autologous serum has been used previously to treat severe dry eye, persistent epithelial defects, and other ocular surface disorders. The main drawbacks are the fact that the patient has to be medically suitable to donate blood, the screening process, and the cost. Also there is a risk that as a blood product, autologous serum could carry unknown infection [Rauz and Saw, 2010]. In this review, 23 studies used fetal calf serum and 7 used autologous serum, with 2 using both (in different patients; Table I). An animal cell and product-free culture system was used in 3 studies, with 2 others using an animal product-free technique for some of the patients in the studies. As well as fetal calf serum and 3T3s, animal products may also be present in culture medium components, such as mouse EGF [Pauklin et al., 2010]. Mariappan et al. [2010] have also recently published an animal-free culture system.

OTHER CULTURE VARIABLES

The culture time varied from 10 to 35 days (Table I). The variation was mainly between studies rather than within. Thus culture time seems to be more down to the techniques used rather than patient factors. The shortest culture time was on contact lenses directly [Di Girolamo et al., 2009]. Shorter culture time was associated with the explant manipulation (shredding or digestion) and a longer time in culture was associated with allogeneic material and the use of airlifting. Airlifting is used by a number of the studies to cause stratification of the epithelium. It is performed by bringing the culture to the air/liquid interface.

GOOD MANUFACTURING PRACTICE AND REGULATIONS

Good manufacturing practice was employed in 4 studies [Shortt et al., 2008; Colabelli Gisoldi et al., 2010; Kolli et al., 2010; Rama et al., 2010]. Cultured limbal epithelial cells for transplantation are now classified as investigational medical products and in the UK they are regulated by the Medicine and Healthcare Products Regulatory Agency (MHRA) in compliance with Tissues and Cells Directive 2004/23/EC, article 1 of Directive 2001/83/EC, article 2 of Regulations No. 1394/2007, amending Directive 2001/83/EC, and Regulations (EC) No. 726/2004. The MHRA has close links to the European Medicines Agency and the US Food and Drug Administration. In the UK and in all other countries in Europe and worldwide, production of cultured human limbal epithelium must be carried out under good medical practice in a specifically licensed laboratory. In order to obtain a licence, the whole production process (including raw materials, manufacturing, supply, and storage) must be assessed and approved and there must be stringent ongoing quality control and inspections [MHRA, 2007]. These new regulations impact on this field of research because in order to obtain a licence, a huge input of validation, time, and funding is required. The cost of treating each patient will also rise due to this.

SURGICAL ASPECTS

TRANSPLANTATION OF CULTURED CELLS

Essentially, the surgical technique is based on the same principle for all studies. Initially, the abnormal conjunctival epithelium is removed from the cornea. At this stage in some cases, Mitomycin C is applied briefly. Then the cultured cells are secured in place. When they are cultured on human amniotic membrane, this needs to be sutured in place, usually just outside the limbus and/or to the resected conjunctival edge peripherally. When fibrin is used as a substrate, it adheres directly to the bare cornea and Di Girolamo et al. [2009] who employ the contact lens for culture simply insert the cell-bearing contact lens. At the same time as cultured limbal epithelial transplantation, 15 patients had keratoplasties, and 3 had other limbal grafts.

PROTECTION OF TRANSPLANTED CELLS

Different methods are used to protect the transplanted cells. A bandage contact lens alone is used in 13 studies, 2 studies used suture closure of the lids (tarsorrhaphy), and 1 study used tape closure of the lids. In 8 studies an extra human amniotic membrane was sutured over the transplant and in 5 studies this was combined with techniques of protection (2 botulinum toxin-induced ptosis, 2 bandage contact lenses, and 1 lateral tarsorrhaphy). Interestingly, Sangwan et al. [2006] stopped the use of bandage contact lenses as it was not felt to be of clinical benefit. However, Shortt et al. [2008] reported a case in which the graft was lost on the first post-operative day, despite using a bandage contact lens. Only 2 studies did not use any method to protect the transplant and in 1 study the aspect of transplant protection methods was not mentioned [Sangwan et al., 2003; Ang et al., 2007; Di Iorio et al., 2010].

POST-OPERATIVE MEDICATION

In the articles, the amount of detail regarding post-operative medication varied considerably. Of note, however, was the use of immune suppression that was used in almost all patients who received an allogeneic graft. Kawashima et al. [2007] used it for all patients, even those who received autografts. All immune suppression regimes used cyclosporin; in 5 studies this was combined with cyclophosphamide. Only one patient was put on mycophenolate mofetil alone and a few other patients did not receive immune suppression, such as the 3-year-old patient. Where duration of immune suppression was reported, it varied from 1 to 12 months. Daya et al. [2005] have argued that from the lack of evidence of detectable donor DNA on the corneal surface after 9 months of surgery, systemic immune suppression is not necessary beyond that period.

ASSESSMENT OF OUTCOMES

The presence of at least some clinical outcomes was one inclusion criterion for this review. All studies described clinical success as a function of examination findings to a lesser or greater extent. In addition all studies except one gave details of visual outcome.

OBJECTIVE CLINICAL ASSESSMENT

Various methods of objective scoring of limbal stem cell deficiency were used in 8 studies. For example, Baradaran-Rafii et al. [2010] used a scoring system from 0 to 4 whereby 2 examiners used slit-lamp examination and clinical photographs to grade epithelial transparency and superficial vascularization. Similar techniques were described in other articles.

CONFOCAL MICROSCOPY

This technique was used in only 1 study that has been used in this review [Shortt et al., 2008]. In future, it would be desirable to see this more widely used for diagnosis and as an outcome measure for limbal stem cell deficiency as it provides a method of directly examining cells in vivo.

POST-OPERATIVE CORNEAL CYTOLOGICAL AND HISTOLOGICAL ANALYSIS

Although corneal impression cytology is used in 10 studies to confirm the diagnosis of limbal stem cell deficiency pre-operatively, it is only used in 7 studies as part of follow-up after the transplant. In 9 studies, corneal tissue removed at the time of subsequent corneal transplantation, is analyzed by histological and immunohisto-chemical techniques for the presence of a normal corneal phenotype.

PATIENT REPORTED OUTCOMES AND QUALITY OF LIFE QUESTIONNAIRES

Only 10 studies give any mention to patient symptoms at all. Formal assessments of patient symptoms are only made in 2 studies. Di Girolamo et al. [2009] used the global symptom score and the mean value reduced from 13 (out of 15) to just 0.3 after treatment. The facial expression analog score reduced from 6 (out of 9) to 2.3. Kolli et al. [2010] used visual analog scores for pain and vision impairment. The mean pain score (on a scale from 0 to 10) significantly reduced from 7.25 to 0.75 after treatment. The subjective visual impairment score also reduced significantly from 7.63 to 3.00. In these instances, improvement in patient reported outcomes appear more dramatic than clinical measures.

Quality of life assessment is also an important outcome measure for two reasons. Firstly, for any condition it is important to consider a holistic view for patients, including physical, emotional, and socioeconomic aspects as well as the pain and disease symptoms [Fitzpatrick et al., 1992]. Secondly, in any healthcare system where there are limited resources, appropriate quality of life measures are essential to justify resource allocation [Spiegelhalter et al., 1992]. Quality of life measures should ideally be multi-factorial, valid, reliable, sensitive to change, and practical to administer [Fitzpatrick et al., 1992]. The quality of life for patients who have had conventional limbal transplants have been reported by Miri et al. [2010]. Even a very modest improvement in measured acuity may mean a great improvement in function. The NEI-VFQ-25 questionnaire was used to compare quality of life outcomes before and after (non-cultured) allogeneic or autologous limbal tissue transplants [Mangione et al., 2001]. The results showed significant improvement in Part 1 "General health and vision" and Part 2 "Difficulty with activities" as well as in an additional question "walking in the street." Part 3 "Responses to visual problems" and other additional questions were not significantly different.

CLINICAL OUTCOMES

SUCCESS RATES

As can be seen from the description on outcome measures above, the outcome parameters varied between the studies reviewed here. Some describe success as an improved corneal surface clinically, whereas others consider success on the basis of more objective parameters, such as visual acuity. In 15 studies, the success rate is quoted as 100% (Table II). All of these studies have less than 10 patients except Sangwan et al. [2005] who reported success in all 15 of the patients. In 11 of the studies with more than 10 patients, the success rate is reported between 59% and 80%. The overall success rate is 76% (77% for autografts and 73% for allografts) at the time of this review and this is similar to that found by Shortt et al. [2007]. Articles that included Kaplan–Meier survival charts appeared to show that failures mainly happened in the first 1–2 years, and after that time, the success rate remained constant [Sangwan et al., 2006; Pauklin et al., 2010; Rama et al., 2010].

TABLE II. Clinical Results

	Autograft success	Allograft success	Total success	2 lines improvement	Subsequent surgery	Complications	Follow-up (months) Mean	Follow-up (months) Range
Ang et al. [2007]	–	100% (1/1)	100% (1/1)	0% (0/1)	–	–	48	–
Baradaran-Rafii et al. [2010]	88% (7/8)	–	88% (7/8)	63% (5/8)	KP (4)	Perforation (1)	34	6–48
Colabelli Gisoldi et al. [2010]	83% (5/6)	–	83% (5/6)	83% (5/6)	KP (4), Cataract (1)	–	24	11–34
Daya et al. [2005]	–	70% (7/10)	70% (7/10)	33% (3/9)	KP (5), Cataract (1), KLAL (5)	Infective keratitis (1)	28	12–50
Di Girolamo et al. [2009]	100% (2/2)	–	100% (2/2)	50% (1/2)	–	–	10.5	8–13
Di Iorio et al. [2010]	80% (133/166)	–	80% (133/166)	–	KP (33)	–	–	6+
Fatima et al. [2007]	100% (1/1)	–	100% (1/1)	100% (1/1)	KP (1)	–	37	–
Grueterich et al. [2002]	100% (1/1)	–	100% (1/1)	100% (1/1)	KP (1), Cataract (1)	–	21	–
Kawashima et al. [2007]	100% (2/2)	100% (4/4)	100% (6/6)	67% (4/6)	KP (6), Cataract (5)	CRVO (1)	32	20–44
Koizumi et al. [2001a]	–	77% (10/13)	77% (10/13)	38% (5/13)	–	Rejection (3), infection (1), conj invasion (2), conj fibrosis (1)	11	6–13
Koizumi et al. [2001b]	–	100% (3/3)	100% (3/3)	0% (0/2)	–	–	6	–
Kolli et al. [2010]	100% (8/8)	–	100% (8/8)	63% (5/8)	KP (1), Redo limbal graft (1)	–	19	12–30
Meller et al. [2009]	–	100% (1/1)	100% (1/1)	100% (1/1)	–	Perforation (1)	31	–
Nakamura et al. [2003]	–	100% (3/3)	100% (3/3)	33% (1/3)	–	–	13	12–14
Nakamura et al. [2004]	100% (1/1)	–	100% (1/1)	100% (1/1)	–	–	19	–
Nakamura et al. [2006]	100% (2/2)	100% (7/7)	100% (9/9)	67% (6/9)	–	Infective keratitis (1)	14.6	6–20
Pauklin et al. [2010]	77% (23/30)	50% (7/14)	68% (30/44)	73% (32/44)	KP (8), Cataract (5)	Bleeding (1), perforation (2)	28.5	9–72
Pellegrini et al. [1997]	100% (2/2)	–	100% (2/2)	50% (1/2)	KP (1)	–	–	24+
Rama et al. [2001]	78% (14/18)	–	78% (14/18)	33% (6/18)	KP (3)	Persistent inflammation + bleeding (4)	17.5	12–27
Rama et al. [2010]	68% (73/107)	–	68% (73/107)	54% (61/107)	KP (62), PTK (2)	Bleeding (12), inflammation (59), herpetic keratitis (3) blepharitis + epitheliopathy (35), residual fibrin (11)	35	12–120
Sangwan et al. [2003]	100% (2/2)	–	100% (2/2)	50% (1/2)	–	Focal recurrence (1)	12	–
Sangwan et al. [2005]	100% (11/11)	100% (4/4)	100% (15/15)	87% (13/15)	KP (15)	Rejection (4—two complete), glaucoma (1)	15.3	7–24
Sangwan et al. [2006]	73% (57/78)	–	73% (57/78)	37% 18/49)	KP (19)	Phthisis (2), keratitis (2), uncontrolled glaucoma (2)	18.3	3–40
Schwab [1999]	76% (13/17)	50% (1/2)	74% (14/19)	16% (3/19)	Redo limbal graft (1)	–	10.5	2–24
Schwab et al. [2000]	60% (6/10)	100% (4/4)	71% (10/14)	36% (5/14)	KP (1)	Epithelial loss (1), cyclosporin related (2) infectious keratitis (1), pyogenic granuloma (1)	13	6–19
Shimazaki et al. [2007]	86% (6/7)	50% (10/20)	59% (16/27)	48% (13/27)	KP (8), Limbal transplants (3)	Infection (1), Ulceration (4), Perforation (4)	29.3	6–85
Shortt et al. [2008]	78% (7/9)	71% (5/7)	75% (12/16)	22% (2/9)	Redo limbal graft (1)	Infective keratitis (1), cyclosporin-related (1), graft detached (1)	9.3	6–13
Tsai et al. [2000]	100% (3/3)	100% (3/3)	100% (6/6)	50% (3/6)	–	–	15	12–18
	77% (373/485)	73% (70/96)	76% (443/581)	51% (197/383)		Overall mean	24	3–120

Success rates quoted by studies are shown as numbers and percentages. They are subdivided by allografts and autografts. Note: 16 eyes lost to follow-up, visual acuity data missing for 198 eyes.
KP, keratoplasty; KLAL, keratolimbal allograft; PTK, phototherapeutic keratectomy.

SUCCESS RATE BY CAUSE OF LIMBAL STEM CELL DEFICIENCY

The success rates can also be subdivided by cause of limbal stem cell deficiency (Table III). According to the pooled results, the success rate for chemical/thermal burns is 75%, congenital causes is 60%, inflammatory disease is 86%, and other causes 80%. Allografts and autografts have a similar success rate of 76%. However, of note, one of the studies with a lower success rate of 59% was not subdivided by cause [Shimazaki et al., 2007]. The results are surprising as it would not be unreasonable to expect that patients with inflammatory eye disease such as Stevens–Johnson syndrome would have a worse outcome than those with a unilateral burn and otherwise relatively normal eyes and who receive autografts.

TABLE III. Results by Etiology of Limbal Stem Cell Deficiency and Source of Donor Material

	Autologous	Allogeneic	Total
Chemical/thermal	75% (195/259)	76% (19/25)	75% (214/284)
Congenital cause	–	60% (9/15)	60% (9/15)
Inflammatory	100% (1/1)	86% (24/28)	86% (25/29)
Other	78% (40/51)	100% (3/3)	80% (43/54)
Total	76% (236/311)	77% (55/71)	76% (291/382)

Note: data not available for 215 eyes.

This finding could related to the use of systemic immunosuppression. Alternatively, it may be due to selection bias of patients who undergo cultured limbal epithelial transplantation as patients at higher risk of failure due to severity of disease may be avoided. Also, the number of patients with chemical/thermal burns is far greater than for the other causes, and studies with fewer patients on the whole had better success rates. This is shown particularly by Rama et al. [2010], who treated 110 patients with burns, following them up for an average of almost 3 years, and a success rate of 68%. Pauklin et al. [2010] reported the outcomes for mixed causes for 44 eyes and found that the best success rate was for pterygia (91%), followed by chemical injuries receiving autologous grafts (75%), then aniridia (50%), and chemical injuries with allografts (33%). Ptergia, although not classic cases of stem cell deficiency, are included in some case series and appear to have a high success rate. They are included in the ''others'' group in this review.

SUCCESS RATE BY METHOD OF CULTURE

For most studies, it is very difficult to compare which culture methods and study protocols are the most successful in terms of outcomes. A small study quoting 100% success may not have enough power (or stringent enough criteria, or follow-up) to demonstrate a reliable final outcome.

For studies where explant and suspension methods were used within each study, Shimazaki et al. [2007] report a 50% success rate (8 out of 16) using the explant technique, and a 73% (8 of out 11) using suspension. However, there are only small numbers and this difference could be explained by the difference in the underlying conditions between the two groups and the fact that more patients had autologous grafts in the suspension group. Nakamura et al. [2006] performed 2 explant cultures using autologous tissue and 6 suspension cultures using allogeneic tissue. Both methods achieved 100% success. Neither study offers any evidence to favor one method over the other.

Rama et al. [2010] analyzed their clinical results along with the percentage of cells in the culture that stained positive for p63 (a putative marker for limbal stem cells). It was found that those cultures which contained more than 3% $p63^{bright}$ cells (i.e., those that stained intensely for p63) were associated with a 78% success rate clinically. Those that had 3% or less $p63^{bright}$ cells were only successful in 11%.

SUCCESS RATE BASED ON IMPROVEMENT IN VISION

For the purposes of this review, available visual acuity raw data was analyzed where possible (Table II). In order to make a comparison, the number of eyes improving by more than or equal to two lines of Snellen visual acuity was identified. There are different methods of calculating this. In some studies, the Snellen fractions are decimalized and then a 0.1 difference is regarded as one line. It must be noted that since the Snellen visual acuity scale is nonlinear this would represent an overestimation of visual improvement, especially for poor vision. Also, visual acuity is measured in different ways below 20/200. The terms counting fingers and hand movement are very inaccurate and may even be given arbitrary Snellen scores. As a result, for this review, in order to achieve an improvement of two lines of Snellen acuity, the final vision level must be at least 20/200, and the number of actual Snellen lines improved is counted.

By this definition, 51% of eyes receiving cultured limbal epithelial transplants had more than or equal to two lines of visual improvement (Table II). It should be remembered that in cases of partial limbal stem cell deficiency, visual acuity may well be reasonable to begin with and thus even success may not give two lines of visual improvement. Also, if there is stromal scarring, the patient may have restored epithelium and a marked difference in symptoms, but no significant improvement in terms of visual acuity. In most studies, the presence of stromal scarring was not systematically recorded and could more easily be indirectly assessed by the number of patients who had simultaneous (in 15 eyes) or subsequent corneal transplants (in 172 eyes). Corneal scarring and transplantation confound the analysis of results of cultured limbal epithelial transplantation. Ideally vision should be prospectively recorded using LogMAR acuity.

COMPLICATIONS

Complications reported after cultured limbal epithelial transplants include 63 records of inflammation, nearly all in one study [Rama et al., 2010]. There are 17 records of bleeding, 8 cases of ocular perforation, 6 cases of infection, 3 cases of glaucoma, and 3 complications related to cyclosporin (Table II). A statement of ''no complications'' is mentioned in 13 articles. The classification and reporting of complications is very variable. One direct comparison of techniques are made by Shimazaki et al. [2007], where the explant culture technique was associated with more infections, ulcers, and perforations compared to the suspension method, although there were many confounding factors.

FOLLOW-UP

Nearly all patients had more than 6 months follow-up (Table II). There were just 1 or 2 patients within large case series who did not meet this criterion. The average length of follow-up was 24 months. Complete turnover of the corneal epithelium is thought to take 9–12 months [Wagoner, 1997]. As described previously nearly all failures occur within the first 2 years. Thus, ideally when considering outcomes, the minimum length of follow-up should be 24 months.

DISCUSSION

Overall the success rate for cultured limbal epithelial transplantation is 76%. This is based on restoration of the corneal epithelium clinically. Fifty percent also had an improvement of $\geq$2 lines Snellen visual acuity. Although infrequently measured, however, there is potentially greater benefit in patient symptoms and quality of life.

It is difficult to compare the studies above for many reasons. Underlying diagnosis, source of material, culture method, surgery, and post-operative care can all influence the outcome. Variable patient numbers in studies, differing amounts of information in published articles and length of follow-up also mean that comparisons are difficult to make. It is surprising therefore that there do seem to be fairly consistent success rates. These findings have not changed significantly from the previous outcome review conducted 3 years ago by Shortt et al. [2007].

As well as the numbers of patients whose outcomes are published, it is predicted that there are many more that are not. Whilst there is so much uncertainty about so many different variables, it does seem important that until there is sufficient evidence to make informed decisions, data are nevertheless published. Ideally, there should be prospective data collection and standardized assessments and outcomes, with a follow-up of 2 years or more. Objective outcome measures which can be compared between groups are also important. As well as objective clinical scores, LogMAR acuity should be recorded and objective assessment such as impression cytology and/or confocal microscopy should be considered. In addition, patient reported outcomes and quality of life measures are vital to realize and demonstrate the full benefit of cultured limbal epithelial transplantation as part of LSCD management.

With newer and evolving regulations governing the good manufacturing practice production of cells, research groups are faced with having to concentrate on meeting these requirements before embarking on long-term follow-up projects. There are also the challenges of actually improving techniques rather than just proving the established ones. An ideal culture system would include an animal and allogeneic human tissue-free culture method governed by the principles of good manufacturing practice and appropriately licensed. The method should be practical and cost-effective. Pellegrini [2010] call for strict criteria to assess culture quality, especially demonstrating that sufficient stem cells are actually present in the culture.

CONCLUSIONS

Limbal stem cell deficiency is a painful and visually disabling disease. Its management is difficult and complex. The transplantation of cultured limbal epithelium does indeed appear to be a promising treatment modality for limbal stem cell deficiency with an overall success rate of 76%. The field is difficult to analyze due the many variables between studies, including the patient and donor eye selection criteria, the culture methods used, the transplantation technique, and the subjective and objective outcome measures. From reviewing the literature, it can be concluded that although complete standardization may be difficult to achieve, some consensus must be achieved on grading limbal stem cell deficiency, and the outcome parameters assessed (in particular visual acuity). It is envisaged that in the next decade, as critical mass of researchers, patients treated, and studies published is achieved, the field will begin to develop some consensus.

REFERENCES

Ahmad S, Figueiredo F, Lako M. 2006. Corneal epithelial stem cells: Characterization, culture and transplantation. Regen Med 1:29–44.

Ahmad S, Kolli S, Lako M, Figueiredo F, Daniels JT. 2010a. Stem cell therapies for ocular surface disease. Drug Discov Today 15:306–313.

Ahmad S, Osei-Bempong C, Dana R, Jurkunas U. 2010b. The culture and transplantation of human limbal stem cells. J Cell Physiol 225:15–19.

Ang LPK, Sotozono C, Koizumi N, Suzuki T, Inatomi T, Kinoshita S. 2007. A comparison between cultivated and conventional limbal stem cell transplantation for Stevens–Johnson syndrome. Am J Ophthalmol 143:178–180.

Baradaran-Rafii A, Ebrahimi M, Kanavi MR, Taghi-Abadi E, Aghdami N, Eslani M, Bakhtiari P, Einollahi B, Baharvand H, Javadi M-A. 2010. Midterm outcomes of autologous cultivated limbal stem cell transplantation with or without penetrating keratoplasty. Cornea 29:502–509.

Colabelli Gisoldi RAM, Pocobelli A, Villani CM, Amato D, Pellegrini G. 2010. Evaluation of molecular markers in corneal regeneration by means of autologous cultures of limbal cells and keratoplasty. Cornea 29:715–722.

Daya SM, Watson A, Sharpe JR, Giledi O, Rowe A, Martin R, James SE. 2005. Outcomes and DNA analysis of ex vivo expanded stem cell allograft for ocular surface reconstruction. Ophthalmology 112:470–477.

DeSousa J-L, Daya S, Malhotra R. 2009. Adnexal surgery in patients undergoing ocular surface stem cell transplantation. Ophthalmology 116:235–242.

Di Girolamo N, Bosch M, Zamora K, Coroneo MT, Wakefield D, Watson SL. 2009. A contact lens-based technique for expansion and transplantation of autologous epithelial progenitors for ocular surface reconstruction. Transplantation 87:1571–1578.

Di Iorio E, Ferrari S, Fasolo A, Bohm E, Ponzin D, Barbaro V. 2010. Techniques for culture and assessment of limbal stem cell grafts. Ocul Surf 8:146–153.

Dua HS, Azuara-Blanco A. 2000. Limbal stem cells of the corneal epithelium. Surv Ophthalmol 44:415–425.

Dua HS, Gomes JAP, King AJ, Maharajan VS. 2004. The amniotic membrane in ophthalmology. Surv Ophthalmol 49:51–77.

Dua HS, Miri A, Alomar T, Yeung AM, Said DG. 2009. The role of limbal stem cells in corneal epithelial maintenance: Testing the dogma. Ophthalmology 116:856–863.

Fatima A, Vemuganti GK, Iftekhar G, Rao GN, Sangwan VS. 2007. In vivo survival and stratification of cultured limbal epithelium. Clin Exp Ophthalmol 35:96–98.

Fitzpatrick R, Fletcher A, Gore S, Jones D, Spiegelhalter D, Cox D. 1992. Quality of life measures in health care. I: Applications and issues in assessment. BMJ 305:1074–1077.

Grueterich M, Espana EM, Touhami A, Ti S-E, Tseng SCG. 2002. Phenotypic study of a case with successful transplantation of ex vivo expanded human limbal epithelium for unilateral total limbal stem cell deficiency. Ophthalmology 109:1547–1552.

Kawashima M, Kawakita T, Satake Y, Higa K, Shimazaki J. 2007. Phenotypic study after cultivated limbal epithelial transplantation for limbal stem cell deficiency. Arch Ophthalmol 125:1337–1344.

Kenyon KR, Tseng SC. 1989. Limbal autograft transplantation for ocular surface disorders. Ophthalmology 96:709–722, discussion 722–3.

Koizumi N, Inatomi T, Suzuki T, Sotozono C, Kinoshita S. 2001a. Cultivated corneal epithelial stem cell transplantation in ocular surface disorders. Ophthalmology 108:1569–1574.

Koizumi N, Inatomi T, Suzuki T, Sotozono C, Kinoshita S. 2001b. Cultivated corneal epithelial transplantation for ocular surface reconstruction in acute phase of Stevens–Johnson syndrome. Arch Ophthalmol 119:298–300.

Kolli S, Ahmad S, Lako M, Figueiredo F. 2010. Successful clinical implementation of corneal epithelial stem cell therapy for treatment of unilateral limbal stem cell deficiency. Stem Cells 28:597–610.

Mangione CM, Lee PP, Gutierrez PR, Spritzer K, Berry S, Hays RD, National Eye Institute Visual Function Questionnaire Field Test I. 2001. Development of the 25-item National Eye Institute Visual Function Questionnaire. Arch Ophthalmol 119:1050–1058.

Mariappan I, Maddileti S, Savy S, Tiwari S, Gaddipati S, Fatima A, Sangwan VS, Balasubramanian D, Vemuganti GK. 2010. In vitro culture and expansion of human limbal epithelial cells. Nat Protocols 5:1470–1479.

Meller D, Fuchsluger T, Pauklin M, Steuhl K-P. 2009. Ocular surface reconstruction in graft-versus-host disease with HLA-identical living-related allogeneic cultivated limbal epithelium after hematopoietic stem cell transplantation from the same donor. Cornea 28:233–236.

MHRA. 2007. Rules and guidance for pharmaceutical manufacturers and distributors. The Orange guide. London: Pharmaceutical Press.

Miri A, Mathew M, Dua HS. 2010. Quality of life after limbal transplants. Ophthalmology 117:638.

Nakamura T, Koizumi N, Tsuzuki M, Inoki K, Sano Y, Sotozono C, Kinoshita S. 2003. Successful regrafting of cultivated corneal epithelium using amniotic membrane as a carrier in severe ocular surface disease. Cornea 22:70–71.

Nakamura T, Inatomi T, Sotozono C, Koizumi N, Kinoshita S. 2004. Successful primary culture and autologous transplantation of corneal limbal epithelial cells from minimal biopsy for unilateral severe ocular surface disease. Acta Ophthalmol Scand 82:468–471.

Nakamura T, Inatomi T, Sotozono C, Ang LPK, Koizumi N, Yokoi N, Kinoshita S. 2006. Transplantation of autologous serum-derived cultivated corneal epithelial equivalents for the treatment of severe ocular surface disease. Ophthalmology 113:1765–1772.

Osei-Bempong C, Henein C, Ahmad S. 2009. Culture conditions for primary human limbal epithelial cells. Regen Med 4:461–470.

Pauklin M, Fuchsluger TA, Westekemper H, Steuhl K-P, Meller D. 2010. Midterm results of cultivated autologous and allogeneic limbal epithelial transplantation in limbal stem cell deficiency. Dev Ophthalmol 45:57–70.

Pellegrini G, Traverso CE, Franzi AT, Zingirian M, Cancedda R, De Luca M. 1997. Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. Lancet 349:990–993.

Pellegrini G, Rama P, De Luca M. 2011. Vision from the right stem. Trends Mol Med 17:1–7.

Puangsricharern V, Tseng SC. 1995. Cytologic evidence of corneal diseases with limbal stem cell deficiency. Ophthalmology 102:1476–1485.

Rama P, Bonini S, Lambiase A, Golisano O, Paterna P, De Luca M, Pellegrini G. 2001. Autologous fibrin-cultured limbal stem cells permanently restore the corneal surface of patients with total limbal stem cell deficiency. Transplantation 72:1478–1485.

Rama P, Matuska S, Paganoni G, Spinelli A, De Luca M, Pellegrini G. 2010. Limbal stem-cell therapy and long-term corneal regeneration. N Engl J Med 363:147–155.

Rauz S, Saw VP. 2010. Serum eye drops, amniotic membrane and limbal epithelial stem cells—Tools in the treatment of ocular surface disease. Cell Tissue Bank 11:13–27.

Sacchetti M, Lambiase A, Cortes M, Sgrulletta R, Bonini S, Merlo D, Bonini S. 2005. Clinical and cytological findings in limbal stem cell deficiency. Graefes Arch Clin Exp Ophthalmol 243:870–876.

Sangwan VS, Vemuganti GK, Iftekhar G, Bansal AK, Rao GN. 2003. Use of autologous cultured limbal and conjunctival epithelium in a patient with severe bilateral ocular surface disease induced by acid injury: A case report of unique application. Cornea 22:478–481.

Sangwan VS, Matalia HP, Vemuganti GK, Ifthekar G, Fatima A, Singh S, Rao GN. 2005. Early results of penetrating keratoplasty after cultivated limbal epithelium transplantation. [Erratum appears in Arch Ophthalmol. 2006 Oct;124(10):1483]. Arch Ophthalmol 123:334–340.

Sangwan VS, Matalia HP, Vemuganti GK, Fatima A, Ifthekar G, Singh S, Nutheti R, Rao GN. 2006. Clinical outcome of autologous cultivated limbal epithelium transplantation. Ind J Ophthalmol 54:29–34.

Schwab IR. 1999. Cultured corneal epithelia for ocular surface disease. Trans Am Ophthalmol Soc 97:891–986.

Schwab IR, Reyes M, Isseroff RR. 2000. Successful transplantation of bioengineered tissue replacements in patients with ocular surface disease. Cornea 19:421–426.

Schwab IR, Johnson NT, Harkin DG. 2006. Inherent risks associated with manufacture of bioengineered ocular surface tissue. Arch Ophthalmol 124:1734–1740.

Shimazaki J, Higa K, Morito F, Dogru M, Kawakita T, Satake Y, Shimmura S, Tsubota K. 2007. Factors influencing outcomes in cultivated limbal epithelial transplantation for chronic cicatricial ocular surface disorders. Am J Ophthalmol 143:945–953.

Shortt AJ, Secker GA, Notara MD, Limb GA, Khaw PT, Tuft SJ, Daniels JT. 2007. Transplantation of ex vivo cultured limbal epithelial stem cells: A review of techniques and clinical results. Surv Ophthalmol 52:483–502.

Shortt AJ, Secker GA, Rajan MS, Meligonis G, Dart JK, Tuft SJ, Daniels JT. 2008. Ex vivo expansion and transplantation of limbal epithelial stem cells. Ophthalmology 115:1989–1997.

Spiegelhalter DJ, Gore SM, Fitzpatrick R, Fletcher AE, Jones DR, Cox DR. 1992. Quality of life measures in health care. III: Resource allocation. BMJ 305:1205–1209.

Tsai RJ, Li LM, Chen JK. 2000. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. N Engl J Med 343: 86–93.

Wagoner MD. 1997. Chemical injuries of the eye: Current concepts in pathophysiology and therapy. Surv Ophthalmol 41:275–313.