Eye (2009) 23, 1966–1971

www.nature.com/eye

CAMBRIDGE OPHTHALMOLOGY SYMPOSIUM

Surgical rehabilitation following severe ocular burns

SJ Tuft and AJ Shortt

Abstract

Chemical and thermal burns can cause devastating injuries to the anterior segment. The consequences of alkali injuries are notoriously severe due to the rapid penetration of these agents into the ocular tissues. Denaturation of tissue, inflammation, and scarring leads to loss of function. An understanding of the pathogenesis of tissue damage has lead to a rational approach to treatment. Emergency irrigation of the eye is essential and there is a 'window of opportunity' during the first 7–10 days after injury when medical treatment can significantly limit the potentially blinding consequences. The acute injury is followed by early and late reparative phases during which the prognosis can be further improved by surgical intervention. Early surgical intervention is targeted at protecting the ocular surface and encouraging re-epithelisation. Later, surgical treatments are directed at ocular surface reconstruction and restoration of vision. However, before any attempt is made at surface reconstruction, the ocular surface environment must be optimised by division of symblepharon, and correction of lid deformity and trichiasis. If there is conjunctivalisation of the corneal surface, limbal stem cell transplantation can restore a corneal epithelial cell phenotype, and transplantation of *in vitro* amplified corneal epithelial stem cells has been developed as an alternative to keratolimbal transfer techniques. Keratoplasty and cataract surgery may then be necessary to clear the visual axis. Finally, keratoprosthesis is an option for the most severely damaged eyes.
Eye (2009) **23,** 1966–1971; doi:10.1038/eye.2008.414; published online 23 January 2009

Keywords: cornea; alkali injury

Department of Clinical Ophthalmology, Moorfields Eye Hospital, London, UK

Correspondence: SJ Tuft, Clinical Ophthalmology, Moorfields Eye Hospital, 162 City Road, London EC1V 2PD, UK.
Tel: +44 207 253 3411; Fax: +44 207 253 2019.
E-mail: s.tuft@ucl.ac.uk

Received: 8 December 2008
Accepted: 8 December 2008
Published online: 23 January 2009

Proprietary interest: None.
Research funding: None.

This work was presented at the Cambridge Opthalmological Symposium 2008

Introduction

Chemical and thermal burns of the anterior segment have the potential to rapidly cause devastating injuries.[1,2] Alkali injuries can be particularly severe because the basic charged molecules rapidly pass through the corneal epithelium and stroma and into the anterior chamber. The superficial location of the corneal epithelial stem cells at the limbus also makes them liable to damage.[3] Despite recent advances in the techniques of ocular surface reconstruction, medical management of the acutely injured eye still has a crucial role in the management of patients following alkali injury, and it is still a major determinant of the eventual outcome.[4] Appropriate emergency intervention can limit the severity and extent of the injury, and reduce the risk of delayed complications, such as corneal melt, surface epithelial failure, and corneal neovascularisation. The potential to modify the outcome by medical intervention represents a window of opportunity that must be used. Immediate irrigation removes the alkali from the ocular surface, although particulate matter, particularly in the fornices, must be debrided as it may act as a continued reservoir for alkali. The buffer and other excipients of the irrigating fluid and other topical medications should not be phosphate-based, as this can lead to acute corneal calcification.[5,6]

Mechanisms of ocular damage and early intervention

Understanding the mechanism of alkali injury has lead to a rational approach to management. Alkaline agents damage tissues by a variety of mechanisms. Primarily, the hydroxyl ions cause acute saponification and acute lysis of cell membranes, with hydrolysis and denaturation of the proteins of the proteoglycans and collagen of the stroma. At the limbus and in the conjunctiva, this results in a loss of function and ischaemia. Because the corneal limbus is thought to be the site of the corneal epithelial stem cell reserve, the extent of limbal epithelial loss and associated ischaemia is an index of the

subsequent prognosis following alkaline injury. Grading systems based on acute signs of epithelial defect, stromal opacity, and limbal ischaemia have been developed to try to predict the eventual prognosis.[7] However, signs may fluctuate and the extent of superficial epithelial loss over the limbus does not necessarily imply coagulation of the underlying corneal stroma and damage to the stem cell niche. In addition, areas of apparent acute ischaemia may revascularise following appropriate treatment, and the prognosis may improve. Alkali damage to the bulbar and tarsal conjunctiva may be accompanied by coagulation of the posterior lid margin that can result in subsequent posterior displacement of the meibomian orifices and trichiasis, and loss of goblet cells and damage to the lachrymal system may result in dry eye disease.

Penetration of alkali into the corneal stroma may result in loss of keratocytes, endothelial damage, and loss of proteoglycan from the stroma. Collagen shrinkage may cause an acute spike in intraocular pressure,[8,9] whereas fragmentation products of the denatured stroma may be chemotactic to neutrophils,[10] which in turn release mediators that recruit more inflammatory cells.[11] Alkali in the anterior chamber can cause iris ischaemia with a fixed dilated pupil, raised intraocular pressure, or secondary hypotony from damage to the ciliary body. Extremely severe injuries may precipitate acute cataract. If there is coexisting alkali injury to the skin of the face or eyelids, the regional burns unit should be consulted for advice. The early use of topical ascorbate and citrate has reduced the incidence of stromal melting following severe burns.[12] The options for early medical treatment are itemised in Table 1.

Table 1 Options for immediate medical treatment of ocular alkali burns

Agent	*Dose*	*Purpose*
Antibiotic	Fluoroquinolone QDS	Prevent infection
Cycloplegic	Cyclopentolate 1% QDS	Control pain
Acetazolamide	250 mg QDS	Control raised intraocular pressure
Steroid[a]	Dexamethasone 0.1% QDS	Reduce inflammation[13]
Doxycycline	100 mg BD	Reduce inflammation and risk of melt
Ascorbate	10% QDS topically, 1 g orally	Prevent collagen lysis[14–16]
Potassium citrate	10% QDS topically	Chelate calcium required by PMNs[17,18]
Lubricant	Hyaluronic acid hourly	Prevent drying

The aim is to promote epithelialisation and prevent ulceration.
All topical agents should be preservative free and without phosphate buffer if possible.
[a]Stop by day 14 if there is persistent epithelial defect to reduce risk of stromal melt.

Surgical intervention is rarely indicated in the acute phase of injury. The conjunctiva and fornices may need to be debrided of necrotic tissue, a process that may require a general anaesthetic for infants and children. An acute amniotic membrane onlay graft may quieten inflammation, conserve limbal stem cells, and encourage epithelialisation.[19] Anterior chamber paracentesis and irrigation with balanced saline have been recommended for the management of severely injured eyes to rapidly reduce the pH of the aqueous,[2,20] although this has not generally been adopted. Paracentesis has also been recommended for acutely elevated intraocular pressure.[2] A therapeutic contact lens or temporary tarsorrhaphy may be required if lid closure is impossible.

Intermediate (postacute) management

Management in the intermediate phase (7–21 days after acute treatment has been initiated) is to control inflammation and to stimulate normal epithelialisation of the ocular surface. Regenerating corneal epithelium may spread from uninjured adjacent areas of limbus, or from peripheral conjunctival epithelium.[21] If the corneal epithelium does not heal due to exposure, dry eye disease, necrotic stroma, or extensive loss of the corneal and conjunctival epithelium, a persistent epithelial defect may develop. This poses a risk of secondary microbial infection, and macrophages recruited by inflammation can lead to stromal melt and neovascularisation. Importantly, corneal melt will stop once epithelialisation is complete. Late stromal calcification may develop as result of a loss of the ability of the damaged stroma to bind calcium from the tear film.

Various strategies may be employed to encourage epithelialisation. A bandage contact lens (eg, silicone hydrogel), a botulinum toxin-induced or surgical tarsorrhaphy, or an amniotic membrane graft will protect the surface. An amniotic membrane can be used as a protective onlay, similar in function to a bandage contact lens, or combined with suturing of the membrane into the conjunctival fornices to prevent scarring and symblepharon. It can also be used as an inlay combined with a superficial keratectomy to remove devitalised superficial stroma to treat persistent epithelial defect. The use of an amniotic membrane onlay has been shown to relieve pain, encourage epithelialisation, and reduce vascularisation and scarring.[22] However, if there is marked inflammation, a membrane may rapidly melt, and a number of repeat surgeries may be necessary. The rapid onset of stromal melting may indicate a secondary infection that should be investigated and treated,

Table 2 Results of keratolimbal surgery and *in vitro* autograft techniques

Author	*Patients* (n)	*Overall success (%)*	*Alkali injury* (n)[a]	*Autograft success (%)*	*Increased VA ⩾2 lines (%)*	*Mean follow-up (years)*
CLAU						
Kenyon and Tseng[31]	26	95	20	NA	NA	1.5
Santos *et al*[26]	33	80	10	80	NA	4
Meallet *et al*[36]	5	100	3	100	100	2
Yao *et al*[37]	39	74	39	74	77	2.3
In-vitro *amplification*						
Pellegrini *et al*[34]	2	100	2	100	100	2
Schwab[38]	19	83	2	50	NA	0.8
Tsai *et al*[39]	6	100	3	100	100	15
Schwab *et al*[40]	14	60	3	33	33	1.1
Rama *et al*[41]	18	78	18	78	72	1.7
Grueterich *et al*[42]	1	100	1	100	100	1.7
Nakamura *et al*[43]	3	100	3	100	100	1
Sangwan *et al*[44]	2	100	2	100	0	1
Nakamura *et al*[45]	2	100	1	100	100	1.5
Shortt *et al*[46]	3	33	3	33	33	0.5

In most publications, the results of grafts for alkali burns are not considered separately. Various different criteria are used to define success.
[a]Eyes after chemical or thermal injury treated with an autograft.

whereas an application of tissue glue can stop melting by excluding the inflammatory cells and their mediators.[23] Acute lamellar or penetrating keratoplasty may then be required if there is corneal perforation. The role of systemic steroid or systemic immunosuppression (eg, with cyclosporine or mycophenolate) to suppress conjunctival inflammation at this stage is uncertain.[24] Alternatives to amniotic membrane grafting to promote epithelialisation and prevent melting after severe injuries are tenonplasty, in which a vascularised pedicle of tenon capsule is rotated over the cornea,[25] or a conjunctival flap if sufficient normal bulbar conjunctiva is available. Once a stable epithelial surface has been achieved and inflammation controlled, further options for ocular surface reconstruction can be considered.

Late phase management

Permanent ocular surface damage following alkaline injuries can include conjunctivalisation of the corneal epithelial surface. This is the result of a loss of the limbal stem cell reserve with overgrowth of the cornea with cells of a conjunctival phenotype, confirmed by the presence of goblet cells and expression of cytokeratin 19. Conjunctivalisation of the ocular surface may be associated with keratinisation, particularly of the posterior lid margin if there has been tarsal plate ischaemia. The cornea may also become vascularised with subepithelial fibrosis and the stroma may become calcified or permanently oedematous due to endothelial failure. Secondary glaucoma and cataract may also require treatment.

Before any consideration of ocular surface reconstruction it is essential that every efforts should be made to optimise the ocular surface environment. Ocular surface reconstruction has a very poor prognosis unless this can be achieved, particularly if there is dry eye disease.[26] Surgery may be necessary to eliminate corneal exposure or abrasion of the ocular surface by trichiasis. A delay in graft surgery until any inflammation has subsided improves the prognosis for keratoplasty survival.[27] Importantly, when assessing corneal thickness, stromal calcification may interfere with pachymetry measurements by ultrasound, resulting in a spuriously high reading suggesting endothelial failure.

Surgical options to reconstruct the ocular surface are based on limbal stem cell transplantation.[28] The object is to transfer viable corneal limbal epithelial stem cells to the damaged cornea to restore the phenotype. If the injury is unilateral, then donor tissue may be harvested from the unaffected eye and transplanted to the injured eye, but for bilateral disease cadaveric donor tissue is required.[29] Because an autograft does not face a risk of rejection, it is the benchmark for the potential of this type of surgery. There are two techniques for autograft transplantation. Whole tissue autografts were first described by Thoft[30] in 1982 and developed into conjunctival limbal autograft (CLAU) transplantation by Kenyon and Tseng.[31] Typically, two 8mm segments of the peripheral cornea and conjunctiva, including the limbus, are harvested from the upper and lower cornea of the donor eye. These areas are selected as they are the primary location of the palisades of Vogt that are thought to be especially rich in stem cells.[32] The abnormal

epithelium of the recipient eye is first removed with any subepithelial fibrovascular tissue and lamellar keratoplasty may be performed if there is stromal opacity or calcification, but penetrating keratoplasty may be required if there is endothelial decompensation. The two donor segments are then sutured to the superior and inferior cornea of the recipient eye, and the eye protected by an amniotic membrane onlay graft, a therapeutic contact lens, or a temporary tarsorrhaphy. Alternatively, instead of isolated limbal grafts, a large diameter lamellar graft, including the limbus, has been used.[33] For *in vitro* amplification techniques, a small biopsy of about 2mm width is taken from a palisade-rich segment of the limbus of the unaffected eye.[34] The biopsy may then be placed directly onto a carrier, such as amniotic membrane or a fibrin sheet, or the epithelial cells suspended from the biopsy , and seeded onto the membrane. The biopsy material is then examined for proliferative potential and cultured in the laboratory for 2–4 weeks until a confluent epithelial layer is obtained before transferring the tissue back to the prepared injured eye.

When performing a CLAU, the size of limbal tissue taken from the healthy eye is significantly larger than that take for the *in vitro* amplification techniques. Although it is generally believed that the donor eyes recover well without complication, removal of this amount of limbus is not without risk, and partial limbal stem cell deficiency has been described in a previously normal eye following limbal biopsy for CLAU.[35] Although the size of the biopsy taken for *in -vitro* amplification techniques is much smaller, the primary culture from the donor eye is not always successful, and with both techniques, the transplanted donor tissue may fail. If this occurs, it is possible to take a further biopsy for *in vitro* amplification techniques, but not for CLAU. On the basis of published case series, the relative success of a CLAU compared with *in vitro* amplification techniques appears to be similar (Table 2). There are, to date, no controlled trials comparing these methods.

During *in vitro* amplification, it is thought that only between 2 and 9% of the cultured cells are stem cells.[40,47–50] Unfortunately, a definitive assessment of the number of stem cells in any culture is presently impossible because there is no definitive marker of limbal epithelial stem cells.[51] The long-term fate of these transplanted stem cells is also unclear. Although it is likely that transplanted stem cells in an autograft will survive indefinitely, evidence from studies of conjunctival limbal allograft transplantation or transplantation of *in vitro* amplified allogeneic limbal stem cells indicates a survival of up to 9 months, whereas a successful clinical outcome may persist.[34,52,41,53] Finally, an important implication of *in vitro* amplification techniques are the infrastructure and staffing costs that make it an expensive and time-consuming procedure that is subject to tight regulation.[54] Despite progress in this field, not all severely damaged eyes are suitable for ocular surface reconstruction, and for these patients synthetic corneal implants (eg, keratoprosthesis,[55] osteo-odontokeratoprosthesis[56]) continues to be an option.

Conclusions

Severe ocular burns are uncommon but can have profound psychological, economic, and social consequences for the patient. They predominantly occur in young male subjects and are associated with industrial accidents, domestic use of alkali, and criminal assault.[57–59] The incidence may have reduced in developed countries as a result of Health and Safety legislation regarding the use of protective eyewear. However, despite recent advances, ocular surface reconstruction of the severely affected eye will continue to present a challenge.

References

1 Wagoner MD. Chemical injuries of the eye: current concepts in pathophysiology and therapy. *Surv ophthalmol* 1997; **41**(4): 275–313.
2 Pfister RR, Pfister DR. Alkali injuries of the eye. In: Krachmer JH, Mannis MJ, Holland EJ (eds). *Cornea*, Vol. 1, 2 edn. Elsevier Mosby: Philadelphia, 2005, pp 1285–1293.
3 Cotsarelis G, Cheng SZ, Dong G, Sun TT, Lavker RM. Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. *Cell* 1989; **57**(2): 201–209.
4 Kuckelkorn R, Schrage N, Keller G, Redbrake C. Emergency treatment of chemical and thermal eye burns. *Acta Ophthalmol Scand* 2002; **80**(1): 4–10.
5 Schrage NF, Schlossmacher B, Aschenbernner W, Langefeld S. Phosphate buffer in alkali eye burns as an inducer of experimental corneal calcification. *Burns* 2001; **27**(5): 459–464.
6 Daly M, Tuft SJ, Munro PM. Acute corneal calcification following chemical injury. *Cornea* 2005; **24**(6): 761–765.
7 Bagley DM, Casterton PL, Dressler WE, Edelhauser HF, Kruszewski FH, McCulley JP *et al*. Proposed new classification scheme for chemical injury to the human eye. *Regul Toxicol Pharmacol* 2006; **45**(2): 206–213.
8 Paterson CA, Pfister RR. Ocular hypertensive response to alkali burns in the monkey. *Exp Eye Res* 1973; **17**(5): 449–453.
9 Paterson CA, Pfister RR. Intraocular pressure changes after alkali burns. *Arch Ophthalmol* 1974; **91**(3): 211–218.
10 Pfister RR, Haddox JL, Sommers CI, Lam KW. Identification and synthesis of chemotactic tripeptides from alkali-degraded whole cornea. A study of N-acetyl-proline-glycine-proline and N-methyl-proline-glycine-proline. *Invest Ophthalmol Vis Sci* 1995; **36**(7): 1306–1316.

11 Sotozono C, He J, Matsumoto Y, Kita M, Imanishi J, Kinoshita S. Cytokine expression in the alkali-burned cornea. *Curr Eye Res* 1997; **16**(7): 670–676.
12 Brodovsky SC, McCarty CA, Snibson G, Loughnan M, Sullivan L, Daniell M *et al*. Management of alkali burns : a 11-year retrospective review. *Ophthalmology* 2000; **107**(10): 1829–1835.
13 Davis AR, Ali QK, Aclimandos WA, Hunter PA. Topical steroid use in the treatment of ocular alkali burns. *Br J Ophthalmol* 1997; **81**(9): 732–734.
14 Levinson RA, Paterson CA, Pfister RR. Ascorbic acid prevents corneal ulceration and perforation following experimental alkali burns. *Invest Ophthalmol* 1976; **15**(12): 986–993.
15 Pfister RR, Paterson CA, Hayes SA. Topical ascorbate decreases the incidence of corneal ulceration after experimental alkali burns. *Invest Ophthalmol Vis Sci* 1978; **17**(10): 1019–1024.
16 Pfister RR, Paterson CA. Additional clinical and morphological observations on the favorable effect of ascorbate in experimental ocular alkali burns. *Invest Ophthalmol Vis Sci* 1977; **16**(6): 478–487.
17 Pfister RR, Nicolaro ML, Paterson CA. Sodium citrate reduces the incidence of corneal ulcerations and perforations in extreme alkali-burned eyes—acetylcysteine and ascorbate have no favorable effect. *Invest Ophthalmol Vis Sci* 1981; **21**(3): 486–490.
18 Pfister RR, Haddox JL, Dodson RW, Deshazo WF. Polymorphonuclear leukocytic inhibition by citrate, other metal chelators, and trifluoperazine. Evidence to support calcium binding protein involvement. *Invest Ophthalmol Vis Sci* 1984; **25**(8): 955–970.
19 Meller D, Pires RT, Mack RJ, Figueiredo F, Heiligenhaus A, Park WC *et al*. Amniotic membrane transplantation for acute chemical or thermal burns. *Ophthalmology* 2000; **107**(5): 980–989; discussion 990.
20 Paterson CA, Pfister RR, Levinson RA. Aqueous humor pH changes after experimental alkali burns. *Am J Ophthalmol* 1975; **79**(3): 414–419.
21 Dua HS, Forrester JV. The corneoscleral limbus in human corneal epithelial wound healing. *Am J Ophthalmol* 1990; **110**(6): 646–656.
22 Kheirkhah A, Johnson DA, Paranjpe DR, Raju VK, Casas V, Tseng SC. Temporary sutureless amniotic membrane patch for acute alkaline burns. *Arch Ophthalmol* 2008; **126**(8): 1059–1066.
23 Vote BJ, Elder MJ. Cyanoacrylate glue for corneal perforations: a description of a surgical technique and a review of the literature. *Clin Experiment Ophthalmol* 2000; **28**(6): 437–442.
24 Den S, Sotozono C, Kinoshita S, Ikeda T. Efficacy of early systemic betamethasone or cyclosporin A after corneal alkali injury via inflammatory cytokine reduction. *Acta Ophthalmol Scand* 2004; **82**(2): 195–199.
25 Kuckelkorn R, Schrage N, Reim M. Treatment of severe eye burns by tenonplasty. *Lancet* 1995; **345**(8950): 657–658.
26 Santos MS, Gomes JA, Hofling-Lima AL, Rizzo LV, Romano AC, Belfort Jr R. Survival analysis of conjunctival limbal grafts and amniotic membrane transplantation in eyes with total limbal stem cell deficiency. *Am J Ophthalmol* 2005; **140**(2): 223–230.
27 Kramer SG. Late numerical grading of alkali burns to determine keratoplasty prognosis. *Trans Am Ophthalmol Soc* 1983; **81**: 97–106.
28 Shortt AJ, Secker GA, Notara MD, Limb GA, Khaw PT, Tuft SJ *et al*. Transplantation of *ex vivo* cultured limbal epithelial stem cells: a review of techniques and clinical results. *Survey of ophthalmology* 2007; **52**(5): 483–502.
29 Solomon A, Ellies P, Anderson DF, Touhami A, Grueterich M, Espana EM *et al*. Long-term outcome of keratolimbal allograft with or without penetrating keratoplasty for total limbal stem cell deficiency. *Ophthalmology* 2002; **109**(6): 1159–1166.
30 Thoft RA. Indications for conjunctival transplantation. *Ophthalmology* 1982; **89**(4): 335–339.
31 Kenyon KR, Tseng SC. Limbal autograft transplantation for ocular surface disorders. *Ophthalmology* 1989; **96**(5): 709–722; discussion 722–703.
32 Shortt AJ, Secker GA, Munro PM, Khaw PT, Tuft SJ, Daniels JT. Characterization of the limbal epithelial stem cell niche: novel imaging techniques permit *in vivo* observation and targeted biopsy of limbal epithelial stem cells. *Stem Cells* 2007; **25**(6): 1402–1409.
33 Vajpayee RB, Thomas S, Sharma N, Dada T, Tabin GC. Large-diameter lamellar keratoplasty in severe ocular alkali burns: A technique of stem cell transplantation. *Ophthalmology* 2000; **107**(9): 1765–1768.
34 Pellegrini G, Traverso CE, Franzi AT, Zingirian M, Cancedda R, De Luca M. Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. *Lancet* 1997; **349**(9057): 990–993.
35 Jenkins C, Tuft S, Liu C, Buckley R. Limbal transplantation in the management of chronic contact-lens-associated epitheliopathy. *Eye* 1993; **7**(Pt 5): 629–633.
36 Meallet MA, Espana EM, Grueterich M, Ti SE, Goto E, Tseng SC. Amniotic membrane transplantation with conjunctival limbal autograft for total limbal stem cell deficiency. *Ophthalmology* 2003; **110**(8): 1585–1592.
37 Yao YF, Zhang B, Zhou P, Jiang JK. Autologous limbal grafting combined with deep lamellar keratoplasty in unilateral eye with severe chemical or thermal burn at late stage. *Ophthalmology* 2002; **109**(11): 2011–2017.
38 Schwab IR. Cultured corneal epithelia for ocular surface disease. *Trans Am Ophthalmol Soc* 1999; **97**: 891–986.
39 Tsai RJ, Li LM, Chen JK. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. *N Engl J Med* 2000; **343**(2): 86–93.
40 Schwab IR, Reyes M, Isseroff RR. Successful transplantation of bioengineered tissue replacements in patients with ocular surface disease. *Cornea* 2000; **19**(4): 421–426.
41 Rama P, Bonini S, Lambiase A, Golisano O, Paterna P, De Luca M *et al*. Autologous fibrin-cultured limbal stem cells permanently restore the corneal surface of patients with total limbal stem cell deficiency. *Transplantation* 2001; **72**(9): 1478–1485.
42 Grueterich M, Espana EM, Touhami A, Ti SE, Tseng SC. Phenotypic study of a case with successful transplantation of *ex vivo* expanded human limbal epithelium for unilateral total limbal stem cell deficiency. *Ophthalmology* 2002; **109**(8): 1547–1552.
43 Nakamura T, Koizumi N, Tsuzuki M, Inoki K, Sano Y, Sotozono C *et al*. Successful regrafting of cultivated corneal epithelium using amniotic membrane as a carrier in severe ocular surface disease. *Cornea* 2003; **22**(1): 70–71.

44 Sangwan VS, Vemuganti GK, Iftekhar G, Bansal AK, Rao GN. 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* 2003; **22**(5): 478–481.
45 Nakamura T, Inatomi T, Sotozono C, Ang LP, Koizumi N, Yokoi N *et al*. Transplantation of autologous serum-derived cultivated corneal epithelial equivalents for the treatment of severe ocular surface disease. *Ophthalmology* 2006; **113**(10): 1765–1772.
46 Shortt AJ, Secker GA, Rajan MS, Meligonis G, Dart JK, Tuft SJ *et al*. *Ex Vivo* Expansion and Transplantation of Limbal Epithelial Stem Cells. *Ophthalmology* 2008; **115**: 1989–1997.
47 Kim HS, Jun Song X, de Paiva CS, Chen Z, Pflugfelder SC, Li DQ. Phenotypic characterization of human corneal epithelial cells expanded *ex vivo* from limbal explant and single cell cultures. *Exp Eye Res* 2004; **79**(1): 41–49.
48 de Paiva CS, Chen Z, Corrales RM, Pflugfelder SC, Li DQ. ABCG2 transporter identifies a population of clonogenic human limbal epithelial cells. *Stem Cells* 2005; **23**(1): 63–73.
49 Watanabe K, Nishida K, Yamato M, Umemoto T, Sumide T, Yamamoto K *et al*. Human limbal epithelium contains side population cells expressing the ATP-binding cassette transporter ABCG2. *FEBS Lett* 2004; **565**(1–3): 6–10.
50 Budak MT, Alpdogan OS, Zhou M, Lavker RM, Akinci MA, Wolosin JM. Ocular surface epithelia contain ABCG2-dependent side population cells exhibiting features associated with stem cells. *J Cell Sci* 2005; **118**(Pt 8): 1715–1724.
51 Schlotzer-Schrehardt U, Kruse FE. Identification and characterization of limbal stem cells. *Exp Eye Res* 2005; **81**(3): 247–264.
52 Henderson TR, Coster DJ, Williams KA. The long term outcome of limbal allografts: the search for surviving cells. *Br J Ophthalmol* 2001; **85**(5): 604–609.
53 Daya SM, Watson A, Sharpe JR, Giledi O, Rowe A, Martin R *et al*. Outcomes and DNA analysis of *ex vivo* expanded stem cell allograft for ocular surface reconstruction. *Ophthalmology* 2005; **112**(3): 470–477.
54 Daniels JT, Secker GA, Shortt AJ, Tuft SJ, Seetharaman S. Stem cell therapy delivery: treading the regulatory tightrope. *Regen Med* 2006; **1**(5): 715–719.
55 Liu C, Hille K, Tan D, Hicks C, Herold J. Keratoprosthesis surgery. *Dev Ophthalmol* 2008; **41**: 171–186.
56 Falcinelli G, Falsini B, Taloni M, Colliardo P. Modified osteo-odonto-keratoprosthesis for treatment of corneal blindness: long-term anatomical and functional outcomes in 181 cases. *Arch Ophthalmol* 2005; **123**(10): 1319–1329.
57 Klein R, Lobes Jr LA. Ocular alkali burns in a large urban area. *Ann Ophthalmol* 1976; **8**(10): 1185–1189.
58 Morgan SJ. Chemical burns of the eye: causes and management. *Br J Ophthalmol* 1987; **71**(11): 854–857.
59 Kuckelkorn R, Makropoulos W, Kottek A, Reim M. [Retrospective study of severe alkali burns of the eyes]. *Klin Monatsbl Augenheilkd* 1993; **203**(6): 397–402.