Original Article

Tissue-Engineered Epithelium Transplantation for Severe Ocular Surface Burns

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Abstract

Purpose: To evaluate the clinical outcomes of tissue-engineered epithelium transplantation for severe ocular surface burns.

Methods: A retrospective observational case series. From October 2005 to May 2011, 19 eyes of 19 patients with grade IV to VI ocular surface burns (Dua Classification) were treated by autologous transplantation of corneal stem cells cultivated on a fibrin gel membrane, with a mean follow-up of 16.2 months (range 12-36 months) Postoperative corneal surface stability, visual acuity (VA), corneal opacity, and neovascularization were evaluated.

Results: No corneal perforation occurred and the entire corneal surface was free from epithelial defects in all eyes. At the final follow-up visit, visual acuity in 17 eyes was improved after surgery, with 6 eyes achieving a VA of 20/100 or better. The corneal vascularization was obviously reduced in 17 (89.5%) eyes.The corneal opacity was also improved in 12 (63.2%) eyes. All donor eyes remained healthy.

Conclusion: Tissue-engineered epithelium transplantation can promote rapid reepithelialization of the ocular surface, inhibit corneal neovascularization, and improve vision for patients with severe ocular surface burns. (***Eye Science 2013; 28: 24-***)

Keywords: ocular surface; tissue-engineering; eye burns

Severe forms of ocular surface burns are responsible for the conjunctivalisation and neovascularisation of the cornea that is often associated with recurrent corneal erosions[1]. In severe eye burns, extensive conjunctival necrosis can lead to limbal ischaemia[2]. Damage to the limbus leads to limbal stem cell deficiency (LSCD) and subsequent corneal epithelial breakdown[3]. This hallmark of LSCD can be associated with a variable destruction of the corneal stroma that leads to heavy scarring or perforation in the worst cases. Amniotic membrane transplantation (AMT) alone can help to restore the corneal epithelial surface but only for moderate chemical burns[4]. In more severe cases, it will not prevent partial or total LSCD[5]. At present, the prognosis following severe injury has improved as a result of the use of limbal stem cell transplantation. Over the last two decades, the surgical technique has evolved from direct limbal transplantation[6,7] to transplantation of ex vivo cultivated limbal epithelial cells[8], which requires less donor tissue and hence is thought to be safer for the donor eye. Autologous cultivated limbal epithelial transplantation can reconstruct the ocular surface more rapidly and patients can feel more comfortable. Tissue-engineered epithelium transplantation (TET) is an autologous transplantation of corneal stem cells cultivated on a fibrin gel membrane. In this study, TET was employed for the successful ocular surface reconstruction of severe eye burns.

Subjects and methods

General information

A retrospective review was conducted of 19 patients with grade IV to VI ocular surface burns (Dua9 Classification) who had undergone TET. The data were collected from October 2005 to May 2011 in the First Hospital Affiliated to Chinese PLA General Hospital.

The inclusion criteria for this study were as follows: (A) patients with a documented history of chemical or thermal burns (Dua Classification grades IV-VI); (B) patients who underwent tissue-engineered epithelium transplantation; (C) patients with

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a postoperative follow-up time ≥12 months; and (D) time between exposure and presentation ≤ 7 days. The following exclusion criteria were applied: (A) patients who had undergone allogeneic limbal transplantation; and (B) patients who had ocular surface diseases before eye burns.

Nineteen patients consisted of thirteen males and six females with a mean age of 39.1 years (range 21 to 60 years). Preoperative diagnosis included corneal alkali burns (n=10), corneal acid burns (n=5), and corneal thermal burns(n=4). The mean time between exposure and presentation was 5.2 days (range 3 to 7 days). Dua9 proposed a new classification for ocular surface burns based on an 'analogue scale' expressed as the number of clock hours of limbal involvement and percentage of conjunctival involvement, which could be broken down into six grades. When limbal involvement is between 6 and 9 clock hours, associated with conjunctival involvement of between 50% and 75%, this would be classified as grade IV. When limbal involvement is greater than 9 clock hours but less than 12 clock hours, associated with conjunctival involvement of greater than 75% but less than 100%, this would be classified as grade V. When the entire limbus (100%) and entire conjunctiva (100%) are involved, this would be classified as grade VI. Nineteen eyes of 19 patients consisted of 8 eyes with grade IV, 9 eyes with grade V, and 2 eyes with grade VI. After initial first aid therapy consisting of profuse irrigation of the eyes and removal of particulate matter, antibiotic eyedrops and eyegels (0.5% levofloxacin), tropicamide or atropine eyedrops, recombinant human epidermal growth factor eyedrops, and artificial tears without preservatives were administered routinely four times a day in all cases. AMT was performed one week after conventional topical medical therapy. A limbal biopsy specimen from the limbus of the healthy contralateral eye was simultaneously cultivated ex vivo for 12–14 days. All cases had undergone AMT with unfavorable outcomes, which clinically manifested as recurrent or persistent epithelial defects, progressive corneal vascularization, and corneal opacity. All cases were carefully assessed prior to TET, including detailed case history record, visual function, and anterior segment examination. The corneal opacity and neovascularization were recorded by slit-lamp biomicroscopy examination. TET was performed at the 28th day (on average) after the burn incident. This study was approved by the institutional review board of the First Affiliated Hospital of General Hospital of PLA, in Beijing, China. We obtained written informed consent from all individuals.

Preparation of tissue-engineered epithelium

In this study, we used a technique of ex vivo cultivated limbal epithelial cells in a fibrin-based matrix[10,11]. A limbal biopsy specimen of approximately 1×1.5 mm was obtained from the limbus of the healthy contralateral eye and was rinsed three times with phosphate group buffer solution, digested with 0.5 g/L pancreatin for 10 min, and neutralized with 10% DMEM medium containing 10% serum. The tissues were then centrifuged and co-cultured with cells in a Petri dish (35 cm diameter) with 0.5mL cornea culture liquid, which consisted of Epilife culture solution (Cascade Biologics, USA) and cornea growth additive. The culture medium was refreshed every three days. The cultivated corneal epithelial cells (5000–8000 cells) were mixed with 300 μL fibrinogen and blood coagulation factor, centrifuged at 3500 g for 30 min, at 4℃ to form tissue-engineered corneal epithelium (figure 1) to be used within 12–14 days. Plasma components were derived from a fibrinogen-rich cryoprecipitate of human plasma instead of fetal bovine serum. All of the cells cultivated in the fibrin gel stained positively for keratin 3, a marker specific for corneal epithelial differentiation.

Figure 1 Tissue–engineered epithelial sheet in the dish

Tissue-engineered epithelium transplantation

A 360° limbal peritomy was performed and the fibrovascular corneal pannus was carefully removed by blunt dissection. Accurate cauterization of scleral and corneal vessels was performed to avoid stagnation of blood once the fibrin disc was placed onto the corneal wound. The cultivated autologous epithelial sheet was secured onto the corneal surface with 10–0 nylon sutures.

Ocular surface observation

Stability of the corneal epithelium, corneal vascularization, and visual acuity were observed in all patients after transplantation. A neovascularization and corneal opacity scoring system reported previously[12] was used to evaluate each eye by the same examiner after surgery. Scoring of the degree of corneal neovascularization depended on the maximum reach of the invasion of corneal neovascularization: grade 0, no neovascularization; grade 1, maximum reach less than one-third the distance between the limbus and corneal center; grade 2, maximum reach between one-third and two-thirds the distance between the limbus and corneal center; grade 3, maximum reach more than two-thirds the distance between the limbus and corneal center; grade 4, maximum invasion reaching the corneal center. The degree of corneal opacity was scored as follows: grade 0, totally clear; grade 0.5, trace or faint corneal haze; grade 1, mild haze of minimal density; grade 2, moderately dense opacity partially obscuring the pupil; grade 3, severely dense opacity completely obscuring the pupil.

Postoperation management

After surgery, antibiotic eyedrops and/or eyegels (0.5% levofloxacin), and artificial tears without preservatives were administered routinely four times a day. The former were continued, as needed, if an epithelial defect was present. No systemic antibiotics or steroids were administered to any patients.

Statistical analysis

Statistical analysis was performed using the CHISS package. Averaged corneal neovascularization and opacity scores before and after surgery were compared with a t test. The level of significance was taken as $P<0.05$.

Results

The clinical profiles of the 19 cases are shown in table 1. No corneal perforation occurred and the entire corneal surface was free from epithelial defects in all eyes 48 hours after transplantation. All subjects reported feeling more comfortable. Four cases showed a small amount of epithelial defects one week after surgery, but the frequent use of levofloxacin eyedrops was effective. At the final follow-up visit, all corneal surfaces remained stable. None of the patients had to undergo a repeat biopsy. None of the donor eyes developed any clinical signs or symptoms of ocular surface disease, and the donor site healed without scarring in 10–12 days. Visual acuity in 17 eyes was improved after surgery, with 6 eyes achieving a VA of 20/100 or better (cases 1, 3, 6, 10, 14, and 15). The corneal vascularization was reduced in 17 (89.5%) eyes.The corneal opacity was improved in 12 (63.2%) eyes. The averaged corneal neovascularization and opacity scores were decreased significantly after surgery ($P<0.05$) (figure 2).

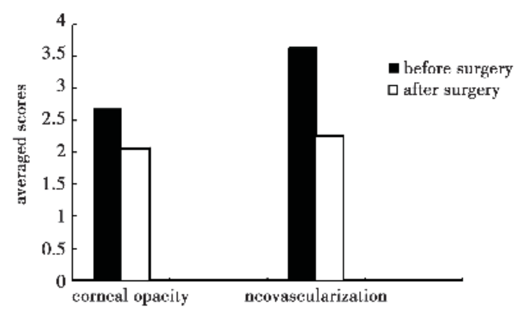

Figure 2 Bar graph showing averaged corneal opacity and neovascularization scores. These were significantly different statistically when compared with preoperation scores

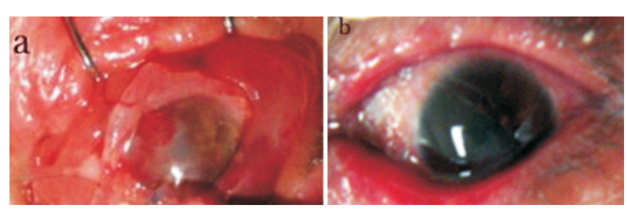

Figure 3 Changes in corneal appearance. patient 14 (grade IV).Before the operation with TET, the corneal surface showed epithelial defects, edema, and opacity (a). Two weeks after TET, the corneal epithelium was stable and the corneal opacity was improved (b)

Discussion

The severity of eye burns varies with the causative agent and the duration of exposure. Management of ocular surface burns requires removal of the inciting

Table 1 Outcome of 19 Eyes That Underwent Tissue-Engineered Epithelium Transplantation

Ratients No.	Age/Gender	Burns			Preoperation			Postoperation			Fellow–up(mos)
		Eye	Agent	Grade	VA	SCO	SCN	VA	SCO	SCN	
1	36/F	Left	thermal	IV	20/300	2	3	20/80	1	2	26
2	45/M	Right	alkali	V	2/200	3	4	20/400	2	2	12
3	52/M	Left	thermal	IV	2/200	2	3	20/100	1	1	13
4	49/M	Left	acid	IV	HM	3	4	2/200	2	3	16
5	30/F	Right	acid	IV	CF	3	4	20/300	2	2	13
6	25/M	Right	thermal	IV	CF	2	3	20/80	1	1	36
7	48/F	Right	alkali	V	HM	3	4	2/200	2	3	13
8	35/F	Left	acid	IV	2/200	3	4	5/200	2	2	24
9	37/M	Left	alkali	VI	CF	3	4	CF	3	4	12
10	50/M	Right	thermal	IV	6/200	2	3	20/100	1	1	13
11	51/F	Right	alkali	V	2/200	3	3	20/400	3	1	22
12	28/M	Left	alkali	V	HM	3	4	CF	3	3	12
13	34/M	Left	alkali	V	LP	3	4	HM	3	3	14
14	30/M	Left	acid	IV	6/200	2	3	20/100	1	1	12
15	60/M	Left	acid	V	2/100	2	3	20/80	1	2	12
16	45/M	Left	alkali	V	6/200	3	4	20/200	2	2	17
17	41/M	Right	alkali	V	LP	3	4	2/200	3	3	15
18	21/M	Right	alkali	V	HM	3	4	2/200	3	3	13
19	25/F	Left	alkali	VI	HM	3	4	HM	3	4	12

F=female; M=male; LP=light perception; HM=hand motions; CF=counting fingers; VA= visual acuity; SCO=scores of corneal opacity; SCN= scores of corneal neovascularization

chemical with an immediate, copious, and prolonged irrigation. In the Dua Classification Grades V and VI, extensive conjunctival necrosis leads to limbal ischemia with a poor prognosis[9]. The amniotic membrane can act as a barrier against the efflux of immune cells, by tempering the immune response and displaying antiangiogenic properties[13]. However, in severe eye burns, reports indicated that AMT needed to be performed several times because the amniotic membrane was dissolved and peeled off from the cornea. The amniotic membrane cannot replace the function of the damaged or dysfunctional limbal stem cells and secure a corneal epithelial phenotype. Nevertheless, AMT can effectively reduce the stromal inflammation for acute eye burns and also provide better conditions for subsequent TET.

The corneal epithelium is continuously renewed by a steady supply of cells from the palisades of Vogt, located at the limbus[14]. Scientific evidence now indicates that adult corneal epithelial stem cells reside at this location[15,16] and the use of auto- or allo-limbal stem cell transplantation has changed the prognosis of severe cases. Previous studies have shown that cultivated limbal epithelial transplantation is as effective as direct limbal transplantation for the treatment of LSCD, while requiring less donor limbal tissue and hence being safer for the donor eye[6–8,17–18]. Autologous cultivated limbal epithelium transplantation without immunosuppression can successfully regenerate the corneal epithelium and reduce vascularization, thus leaving open the possibility of a penetrating keratoplasty that would increase visual acuity in the future[19–22]. Recently, Hirayama et al reported[23] that a better midterm clinical outcome was achieved with cultivated oral mucosal epithelial cell sheet transplantation (COMET) using fibrin-coated culture dishes as compared with COMET using AM as a substrate for treating severe stem cell deficiency.

We cultivated limbal epithelial cells ex vivo in a fibrin-based matrix without AM carrier and a 3T3 fibroblast layer. All cell strains were cultured in a cornea growth medium with added human corneal growth supplements. The cultured corneal epithelial stem cells were suspended in a fibrin gel cross-linked by factor XIII. Plasma components were derived from a fibrinogen-rich cryoprecipitate of human plasma instead of fetal bovine serum, thereby avoiding the risks associated with using animal-derived products for culture. Identification of the inner and outer layer of the cultivated autologous epithelial sheet by surgeons was not necessary while TET was performed. After surgery, no administration of immunosuppressive therapy is needed.

Currently, opinion is divided about which of the two options-conjunctival limbal autografting (CLAU) and cultivated limbal epithelial transplantation (CLET)-is the better surgical alternative. Proponents of CLAU consider ex vivo cultivation unnecessary and expensive, while proponents of CLET consider that autografting is technically risky and that CLET is a technique that allows less than one clock hour of donor limbus to be expanded ex vivo into a transplantable epithelial sheet[24]. Although an effective technique for autologous transplantation of corneal stem cells cultivated on fibrin gel membrane is desirable, no large studies have yet reported on the long-term outcomes of TET.

In this study, all cases received standard medical treatment and AMT, but the ocular surface continued to show recurrent or persistent epithelial defects. TET was performed once the ocular surface inflammation had been controlled. All donor eyes remained healthy. No complications occurred in the healthy contralateral eyes where the biopsy was taken, suggesting the safety of this surgical procedure. After TET, complete epithelialization was achieved in all the eyes. The tissue-engineered epithelial sheet has a relatively even thickness. TET without immunosuppression can successfully recreate the ocular surface to prevent corneal perforation and reduce vascularization and corneal opacity (figure 3) in place of lamellar corneal transplantation, thereby providing a basement for subsequent keratoplasty for increasing visual acuity later. The human corneal epithelium is renewed approximately every 9 to 12 months20 and long-term follow-up times are therefore needed. Our clinical outcomes were assessed at least 1 year after TET. At the final follow-up visit, the corneal neovascularization was lessened in 89.5% of the eyes and opacity was improved in 63.2% of the eyes. The neovascularization and corneal opacity scores were significantly lower after TET than before ($P<0.05$). Visual acuity in 17 of 19 eyes was improved, with 6 eyes achieving a VA of 20/100 or better. We found that the results of treatment correlated with burn degree and causative agent. In a severe alkali burn or a Grade VI burn, the postoperative visual acuity was poor.

In summary, this study evaluated the clinical outcomes of TET for severe ocular surface burns. Some debate still continues regarding the timing of limbal transplantation and the actual mechanism by which limbal transplantation works. Further studies are required in order to determine whether this treatment replenishes the stem cell reserve or revives the surviving stem cells by improving their microenvironment.

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