Treatment of limbal stem cell deficiency with autologous cultivated oral mucosal epithelial transplantation

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Research article

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Abstract

Background: To evaluate the feasibility of autologous cultivated oral mucosal epithelial transplantation (COMET) for the treatment of limbal stem cell deficiency (LSCD).

Methods: Seven eyes from seven different patients with monocular LSCD were included in this study. Autologous oral mucosal epithelial cells were fabricated on ex vivo using amniotic membranes as a substrate. Clinical efficacy was evaluated by the coefficient of best-corrected visual acuity (BCVA). Clinical formation of the conjunctiva and symblepharon was evaluated and graded on a scale from 0 to 3. Clinical safety was evaluated by the presence of persistent epithelial defects, infection, and ocular hypertension.

Results: Autologous COMET was successfully performed in all seven patients. The mean follow-up period was 10.7 months, during which time the postoperative formation of the conjunctiva and symblepharon was inhibited. BCVA was improved more than two lines in six eyes (86%) during the follow-up period. Complete reepithelialization of the corneal surfaces occurred in all treated eyes. No persistent epithelial defects, corneal infection, or postoperative ocular hypertension were observed.

Conclusions: Autologous COMET offers a viable and safe alternative for the reconstruction of a stable ocular surface and improves vision in patients with LSCD.

Background

Corneal epithelial stem cells reside in the basal layer of the limbus and regulate the renewal of the corneal epithelium [1]. When epithelial stem cells become severely damaged by inflammation or trauma, the sources of corneal epithelial cells can become depleted. The conjunctival epithelium then invades the corneal surface where epithelial defects persist, resulting in severe visual impairment. Such characteristics are typical of limbal stem cell deficiency (LSCD) [2–3].

In unilateral LSCD, autologous transplantation of limbal tissues obtained from the healthy eye can be used [4–6]. This procedure requires a large limbal graft from the healthy eye and may result in LSCD in that eye as well [7]. As this approach is therefore not possible in patients with bilateral lesions [8], the harvesting of allogenic grafts becomes necessary, requiring long-term immunosuppression and increasing the risk of serious eye and systemic complications [8–9].

Tissue-engineered epithelial cell transplantation is a treatment option for patients with LSCD [10–14]. Recently, the clinical effectiveness of autologous COMET was reported in patients with bilateral LSCD [11–13]. The major advantage of such an approach is that it avoids the need for postoperative immunosuppression.

We report here the results of ocular surface reconstruction in seven eyes from seven different patients with LSCD using autologous COMET.
Methods

Subjects

This study was approved by the institutional review board of Hangzhou First People’s Hospital, China. Prior informed consent was obtained from all patients. The study included seven eyes from seven different patients with unilateral LSCD who underwent autologous COMET at our hospital from April 2013 to October 2015. Patient 1 had an acute thermal burn and a 2-month-old corneal epithelial defect. Patient 2 had recurrent pterygium. The other five patients had chronic burns that were covered by a fibrovascular ingrowth from the limbus over the central cornea and symblepharon.

Cultivation of oral mucosal epithelial sheets

A 10-by-10-mm oral mucosal biopsy was performed on each patient under local anesthesia. Oral mucosal epithelium was then incubated at 4 °C for 5 h with dispase II (1.25 mg/mL), followed by treatment with trypsin-EDTA for 10 min to create a single-cell suspension. The single-cell suspension was then incubated with oral keratinocyte medium on an amniotic membrane for 2 weeks.

Immunohistology

Cryosections were stained with hematoxylin and eosin, and immunostained with monoclonal anti-keratin 3 antibodies. Incubation with phosphate buffered saline was used as the negative control, and native human corneal and oral mucosal tissues were used as the positive controls.

Surgical procedures were performed by the same surgeon (Dr. Ye). The subconjunctival fibrovascular tissue was removed, and autologous COMET was performed, as previously described [5, 10]. Then the grafted corneal surface was covered with a therapeutic soft contact lens. After surgery, tobramycin and dexamethasone eye drops were applied four times per day, and the dose was then decreased over time. During the first 3 days after surgery, dexamethasone (5 mg per day) was administered intravenously to reduce postoperative inflammation. Both best-corrected visual acuity (BCVA) and tonometry were measured; the ocular surface was also inspected by slit-lamp biomicroscopy and fluorescence staining in all patients every 2 to 4 weeks during the follow-up period, beginning one week after COMET.

Evaluation of clinical efficacy

The clinical results were evaluated and graded on a scale from 0 to 3, as follows [15]:

The extent of conjunctiva formation was graded as follows: 0 = absence of formation, 1 = conjunctiva formation involving less than one-quarter of the corneal surface, 2 = conjunctiva formation involving one-quarter to one-half of the corneal surface, and 3 = conjunctiva formation involving more than one-half of the corneal surface.

The extent of symblepharon formation was scored as follows: 0 = no symblepharon, 1 = formation involving only the conjunctival surface, 2 = formation involving less than one-half of the corneal surface,
and 3 = formation involving more than one-half of the corneal surface.

**Evaluation of clinical safety**

The following postoperative complications were evaluated: persistent epithelial defects lasting more than 4 weeks, infection, and ocular hypertension. Cases of preoperative ocular hypertension were not considered.

**Results**

**Characterization of autologous cultivated oral mucosal epithelial sheets**

Cultivated oral mucosal epithelial cells within one to two cell layers (Fig. 1A) more closely resemble the corneal epithelium (Fig. 1B) than they resemble native oral mucosal epithelium (Fig. 1C). Native oral epithelial cells (Fig. 2A) and corneal epithelial cells (Fig. 2B) express keratin 3, which is a characteristic phenotypic marker; cultivated oral mucosal epithelial cells (Fig. 2C) also express keratin 3.

**Clinical results of autologous COMET**

Cultivated oral mucosal epithelial cells were successfully generated for all seven patients. Complete reepithelialization of the corneal surfaces occurred within 4 days after surgery, as determined by fluorescein staining. Measurements of postoperative visual acuity are summarized in Table 1. The effective coefficient of BCVA was 2.21 ± 0.69. During the follow-up period, BCVA improved more than two lines in six eyes (86%), and the formation of the conjunctiva and symblepharon was significantly inhibited (Table 1 and Fig. 3). All corneal surfaces stabilized during the mean follow-up period of 10.7 months. No instances of ocular hypertension or corneal infection were observed.

Table 1. Clinical results
| Patient follow-up (months) | Last follow-up | Prior to surgery | Last follow-up |
|---------------------------|----------------|-----------------|----------------|
|                           | NCT mmHg       | BCVA            | Conjunctiva    | Symblepharon mmHg | BCVA | Conjunctiva | Symblepharon |
| 1                         | 16             | 13.0            | 0.08           | 1               | 0    | 16.0        | 0.15         | 1             | 0             |
| 2                         | 14             | 15.0            | 0.1            | 3               | 2    | 16.0        | 0.15         | 2             | 0             |
| 3                         | 15             | 7.7             | 0.5            | 1               | 2    | 8.0         | 0.8           | 1             | 0             |
| 4                         | 12             | 13.0            | 0.1            | 3               | 1    | 15.0        | 0.4           | 1             | 0             |
| 5                         | 6              | 11.0            | 0.2            | 2               | 2    | 11.5        | 0.3           | 1             | 0             |
| 6                         | 6              | 14.7            | 0.1            | 3               | 1    | 15.3        | 0.25          | 2             | 0             |
| 7                         | 6              | 13.0            | 0.02           | 3               | 3    | 16.0        | 0.05          | 3             | 2             |

**Discussion**

> Oral mucosal epithelial cells were inoculated onto cell-free amniotic membranes in our study. These cells grew, divided, and maintained their phenotype without the presence of feeder cells, simplifying the cell culture process and preventing exogenous biological contamination [16]. These cells were also able to generate cornea-like epithelium under our culture conditions. The advantage of this type of ex vivo cell expansion over conventional methods is that only a small mucosal biopsy is needed, thus minimizing both damage to the oral cavity and the risk of graft rejection.

Our study shows that autologous COMET was effective for the reconstruction of the corneal and limbal surfaces, as oral mucosal epithelial cells express keratin 3, which is also expressed by the corneal epithelium[7]. These were also essential for the clinical success of COMET in that the subconjunctival fibrovascular tissue was totally removed, the amniotic membrane, which remained very flat, was securely attached to the surface of the eyeball, and the grafted corneal surface was covered with a therapeutic soft contact lens during the early postoperative period. The complete reepithelialization of the corneal surfaces was observed, postoperative visual acuity was improved, and the formation of the conjunctiva and symblepharon was significantly inhibited. These findings indicate that the rapid completion of epithelialization inhibits the proliferation of fibrovascular tissue and lowers the incidence of complications.
There were no instances of ocular hypertension or corneal infection during the postoperative follow-up period because COMET involves autologous transplantation and thus the patients did not require prolonged postoperative immunosuppression. It is therefore a safe and cost-effective surgical procedure for all patients.

**Conclusions**

Autologous COMET does not damage healthy eyes and is particularly suitable for bilateral LSCD. Long-term clinical results of a larger series of patients are needed to assess the efficacy of this type of transplantation.

**List Of Abbreviations**

- BCVA - Best-corrected visual acuity
- COMET - Cultivated oral mucosal epithelial transplantation
- LSCD - Limbal stem cell deficiency

**Declarations**

**Acknowledgements**

Not applicable.

**Authors’ contributions**

YQJ and YFY interpreted this data and drafted the manuscript. HCZ and HMW collected the data and reviewed the literature. All authors approved the final manuscript.

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**Availability of data and materials**

The datasets in this study can be obtained from the corresponding author on reasonably required.

**Ethics approval and consent to participate**

This study was conducted for all patients in Hangzhou First People's Hospital. The study was approved by the Institutional Review Board of Hangzhou First People's Hospital. All participants received written
informed consent.

**Consent for publication**

Written informed consents for publication were obtained from all participants.

**Competing interests**

The authors declare that they have no competing interests.

**References**

1. Thoft RA, Friend J. The X, Y, Z hypothesis of corneal epithelial maintenance. Invest Ophthalmol Vis Sci 1983;24:1442-3.
2. Tseng SC. Concept and application of limbal stem cells. Eye 1989;3:141-57.
3. Nishida K. Tissue engineering of the cornea. Cornea 2003;22 Suppl 7:S28-34.
4. Kenyon KR. Limbal autograft transplantation for chemical and thermal burns. Dev Ophthalmol 1989;18:53-8.
5. 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;109:2011-7.
6. Tsai RJ, Li LM, Chen JK. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. N Engl J Med 2000;343:86-93.
7. Jenkins C, Tuff S, Liu C, Buckley R. Limbal transplantation in the management of chronic contact-lens-associated epitheliopathy. Eye 1993;7(Pt 5):629-33.
8. 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;72:1478-85.
9. Tsubota K, Satake Y, Kaido M, Shinozaki N, Shimmura S, Bissen-Miyajima H, et al. Treatment of severe ocular-surface disorders with corneal epithelial stem-cell transplantation. N Engl J Med 1999;340:1697-1703.
10. Shimazaki J, Aiba M, Goto E, Kato N, Shimmura S, Tsubota K. Transplantation of human limbal epithelium cultivated on amniotic membrane for the treatment of severe ocular surface disorders. Ophthalmology 2002;109:1285-90.
11. Nishida K, Yamato M, Hayashida Y, Watanabe K, Yamamoto K, Adachi E, et al. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. N Engl J Med 2004;351:1187-96.
12. Takahiro N, Kazunori T, Tsutomu I, Sotozono C, Kinoshita S. Long-term results of autologous cultivated oral mucosal epithelial transplantation in the scar phase of severe ocular surface disorders. Br J Ophthalmol 2011;95:942-6.
13. Ang LP, Nakamura T, Inatomi T, Sotozono C, Koizumi N, Yokoi N, et al. Autologous serum-derived cultivated oral epithelial transplants for severe ocular surface disease. Arch Ophthalmol 2006;124:1543-51.

14. Satake Y, Dogru M, Yamane GY, Kinoshita S, Tsubota K, Shimazaki J. Barrier function and cytologic features of the ocular surface epithelium after autologous cultivated oral mucosal epithelial transplantation. Arch Ophthalmol 2008;126:23-8.

15. Sotozono C, Ang LPK, Koizumi N, Higashihara H, Ueta M, Inatomi T, et al. New grading system for the evaluation of chronic ocular manifestations in patients with Stevens–Johnson syndrome. Ophthalmology 2007;114:1294-302.

16. Collins SJ, Lawson VA, Masters CL. Transmissible spongiform encephalopathies. Lancet 2004;363:51-61.

Figures
Figure 1

The epithelial cells were stained with hematoxylin and eosin. Cultivated oral mucosal epithelial cells within one to two cell layers (A, arrow, ×40) more closely resemble the corneal epithelium (B, ×40) than they resemble native oral mucosal epithelium ((C, ×40).
Figure 2

The epithelial cells were immunostained with monoclonal anti-keratin 3 antibodies. Native oral epithelial cells (A, ×40), corneal epithelial cells (B, ×40), and cultivated oral mucosal epithelial cells (C, ×40, arrow) were immunostained with anti-keratin 3 antibodies.
Figure 3

Slit-photo images of seven patients before and after surgery. Prior to COMET, all eyes displayed severe destruction of the ocular surface due to LSCD. The appearance of the eyes postoperatively reveals a relatively smooth, epithelialized corneal surface.