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)	Prior to surgery				Last follow-up			
		NCT	BCVA	Conjunctiva	Symble-	NCT	BCVA	Conjunctiva	Symble-
		mmHg			pharon	mmHg			pharon
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

p>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.

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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, $\times 40$) more closely resemble the corneal epithelium (B, $\times 40$) than they resemble native oral mucosal epithelium ((C, $\times 40$).

Figure 2

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