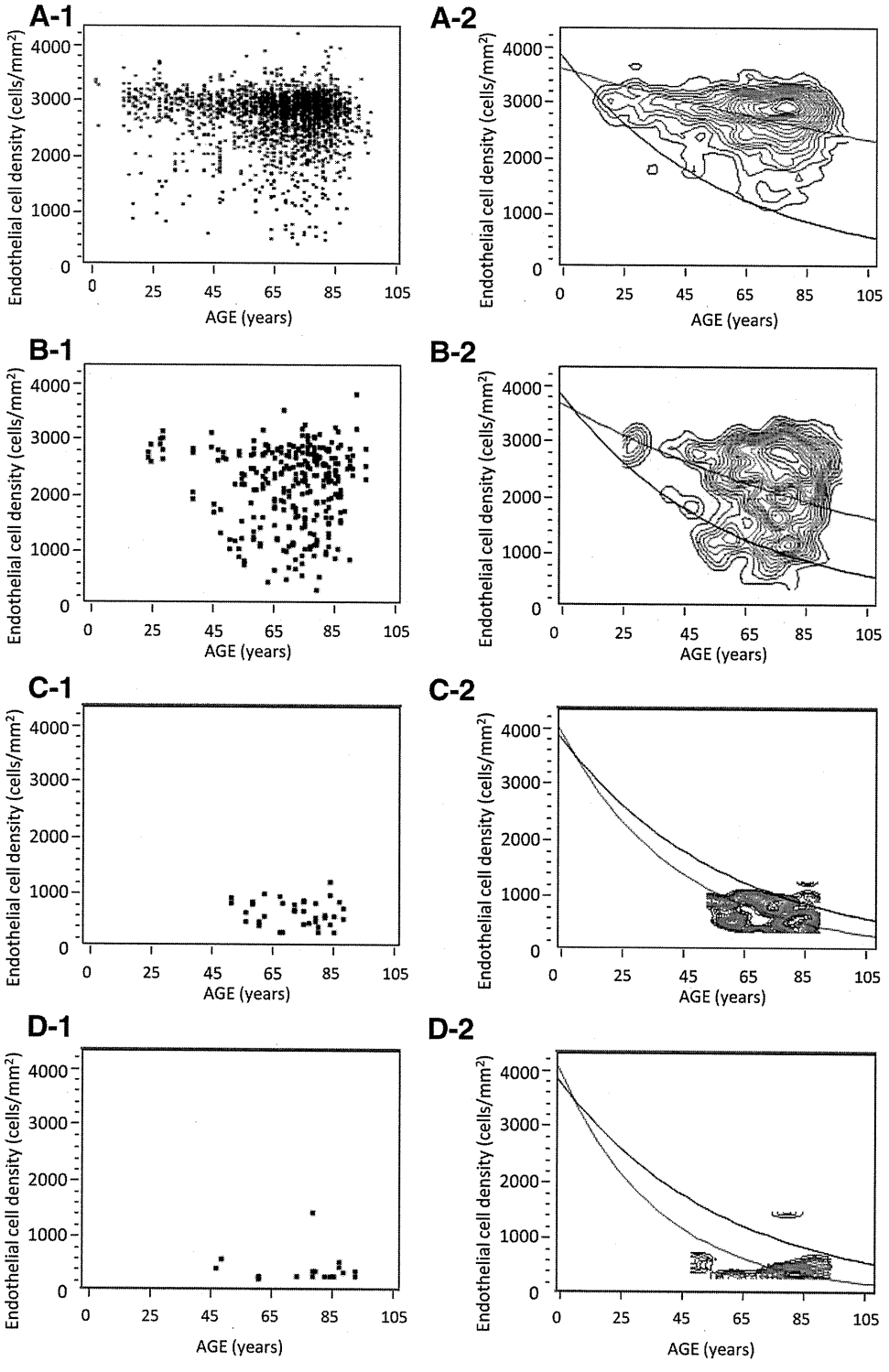


FIGURE 1. Scatterplots (*left*) and contour maps of nonparametric density smoothing (*right*) of each stage. (**A-1**, **A-2**) Stage 0, (**B-1**, **B-2**) stage 1, (**C-1**, **C-2**) stage 2, and (**D-1**, **D-2**) stage 3. *Red curves*: age-ECD curves of each stage calculated by least-squares method. The decrease rates of each stage were 0.44% (stage 0), 0.81% (stage 1), 2.65% (stage 2), and 3.08% (stage 3). The contour maps showed that the age-ECD curve of 2.00% decrease rate ($ECO_{2.0}$, *black curves*) ran through a trough between peaks of all stages. Most of the peaks in stages 0 and 1 were located above $ECO_{2.0}$, whereas peaks of stages 2 and 3 were located below $ECO_{2.0}$.

normal eyes reported in previous studies (Table 3).[10,12–16] Contour maps show that most of the peaks in stage 0 and 1 were located above the age-ECD curve of the 2.00% decrease rate, whereas peaks of stage 2 and 3 were located below this curve. Table 4 shows binary classification based on the age-ECD curve of a 2.00% decrease rate, designated novel ECD cutoff 2 ($ECO_{2.0}$), dividing stages 0+1 and stages 2+3 (Table 4) or stage 1 and stages 2+3 (Table 4). The high sensitivity and specificity of these classifications suggested that $ECO_{2.0}$ is an adequate cutoff between eyes with corneal edema and those without edema.

Age-ECD Curve of 1.4% and 2.0% Divides Stage 1 into Three Distinct Groups

The contour map of stage 1 consisted of several peaks. Figure 2 shows that the age-ECD curve of the 1.40% decrease rate, designated novel ECD-cutoff point 1 ($ECO_{1.4}$), divides these peaks into a high-density group ($>ECO_{1.4}$), and a low-density group ($<ECO_{1.4}$). ANOVA revealed that the age-ECD curves of each group predicted ECD according to age, with statistical significance: The F ratio and P value were 803.3 and <0.0001

TABLE 2. Mean ECD with Sample Sizes at 5-Year Intervals for Grades 0 to 3

	0–9 y		10–14 y		15–19 y		20–24 y		25–29 y	
	Eyes	ECD	Eyes	ECD	Eyes	ECD	Eyes	ECD	Eyes	ECD
Stage 0	4	3073.3 ± 392.6	7	3020.4 ± 330.1	47	2769.2 ± 530.1	31	2837.4 ± 567.3	60	2853.1 ± 507.6
Stage 1	0	—	0	—	0	—	4	2765.0 ± 128.8	6	2954.5 ± 175.6
Stage 2	0	—	0	—	0	—	0	—	0	—
Stage 3	0	—	0	—	0	—	0	—	0	—
	30–34 y		35–39 y		40–44 y		45–49 y		50–54 y	
	Eyes	ECD	Eyes	ECD	Eyes	ECD	Eyes	ECD	Eyes	ECD
Stage 0	58	2732.6 ± 511.3	54	2741.9 ± 324.7	80	2672.2 ± 462.5	99	2687.8 ± 507.8	128	2754.6 ± 370.5
Stage 1	0	—	4	2423.0 ± 474.1	7	2503.7 ± 541.9	7	1934.3 ± 763.9	14	1865.2 ± 703.0
Stage 2	0	—	0	—	0	—	2	881.0 ± 60.8	2	592.0 ± 120.2
Stage 3	0	—	0	—	1	461.0	1	622.0	0	—
	55–59 y		60–64 y		65–69 y		70–74 y		75–79 y	
	Eyes	ECD	Eyes	ECD	Eyes	ECD	Eyes	ECD	Eyes	ECD
Stage 0	195	2701.2 ± 408.1	325	2671.9 ± 464.4	384	2677.7 ± 449.1	494	2698.4 ± 435.0	496	2691.2 ± 421.3
Stage 1	25	2105.2 ± 673.3	28	2219.4 ± 695.5	39	2124.8 ± 743.7	61	2242.5 ± 719.4	44	2159.0 ± 741.7
Stage 2	4	645.8 ± 224.3	2	797.5 ± 282.1	7	562.9 ± 329.5	7	730.7 ± 149.5	7	483.0 ± 183.7
Stage 3	2	284.5 ± 21.9	0	—	0	—	2	302.5 ± 3.5	7	524.0 ± 418.9
	80–84 y		85–89 y		≥90 y					
	Eyes	ECD	Eyes	ECD	Eyes	ECD				
Stage 0	309	2698.9 ± 440.4	116	2624.5 ± 457.3	22	2563.7 ± 299.3				
Stage 1	47	2264.2 ± 556.2	17	2279.2 ± 597.9	5	2962.0 ± 597.1				
Stage 2	7	680.6 ± 318.1	3	723.3 ± 155.7	0	—				
Stage 3	5	302.4 ± 5.4	3	482.3 ± 97.1	2	352.5 ± 74.2				

Eye data are expressed as the number, and the ECD in cells per square millimeter.

in the high-density group and 945.7 and <0.0001 in the low-density group. The decrease rate of the age-ECD curve in the high-density group was 0.56%, which was very close to that of the stage 0 age-ECD curve. On the other hand, the decrease rate in the low-density group was 2.00%, which coincided with $ECO_{2.0}$. These results suggest that the decrease rate of the high-density group in stage 1 was nearly normal, whereas the low-density group in stage 1 was located on the border between eyes with and without corneal edema. We therefore classified stage 1 on the basis of $ECO_{1.4}$ and $ECO_{2.0}$, as follows (Fig. 3):

Stage 1a, asymptomatic guttata cornea (AGC): above $ECO_{1.4}$

Stage 1b, borderline guttata cornea (BGC): between $ECO_{1.4}$ and $ECO_{2.0}$

Stage 1c, preliminary stage of FCD (pre-FCD): below $ECO_{2.0}$

TABLE 3. Decrease Rates of Stage 0 in the Present Study and Normal Unoperated Eyes Reported in the Previous Studies

Author	Decrease Rate (%/y)	Nation
Murphy et al.[10]	0.56	United States
Cheng et al.[12]	1.00	England
Ambrose et al.[13]	0.60	England
Numa et al.[14]	0.30	Japan
Bourne et al.[15]	0.60	United States
Rao et al.[16]	0.30	India
Present study	0.44	Japan

DISCUSSION

To obtain a sufficient number of age-ECD data to compare FCD (stage 2+3), guttata cornea without edema (stage 1), and control group without guttata cornea (stage 0), we performed a retro-

TABLE 4. Binary Classification of Clinical Stage

Clinical Stage	Classification Based On $ECO_{2.0}$		
	Below $ECO_{2.0}$	Above $ECO_{2.0}$	Total
Total Eyes			
Stage 2+3	60	4	64
Stage 0+1	122	3095	3217
Total	182	3099	3281
Sensitivity, %	93.75		
Specificity, %	96.21		
Eyes with Guttata Cornea			
Stage 2+3	60	4	64
Stage 1	27	281	308
Total	87	285	372
Sensitivity, %	93.75		
Specificity, %	91.23		

Data are based on the age-ECD curve of 2.00% decrease rate as a novel ECD-cut-off ($ECO_{2.0}$), sensitivity and specificity to detect stage 2+3 from total eyes or the eyes with guttata cornea based on the classification.

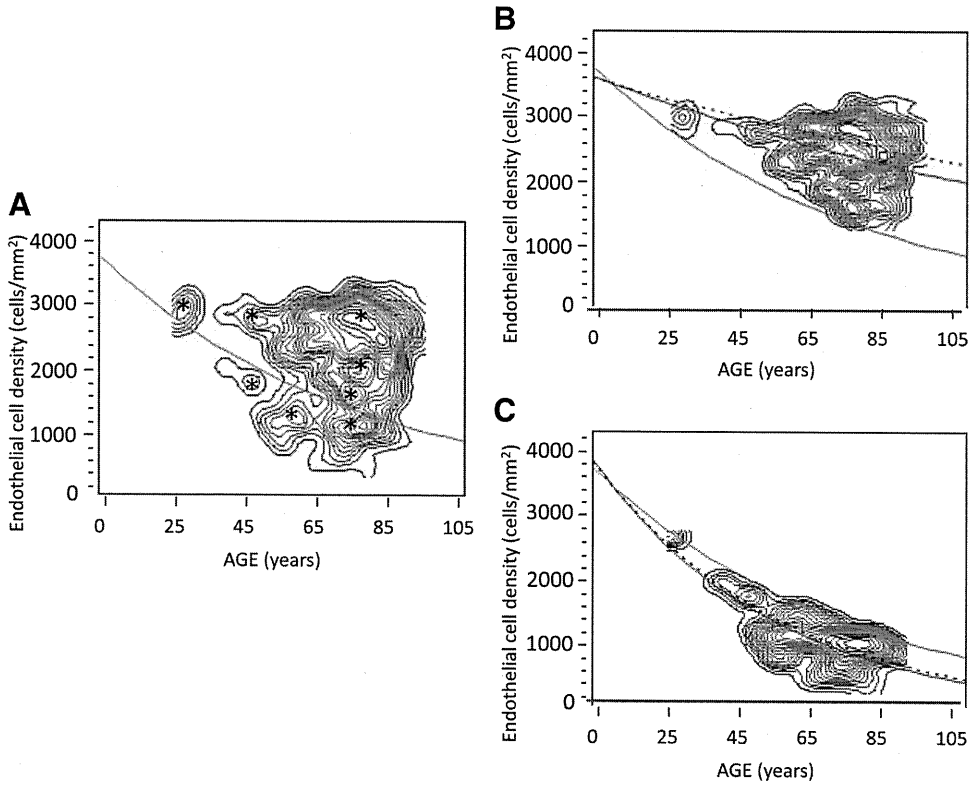
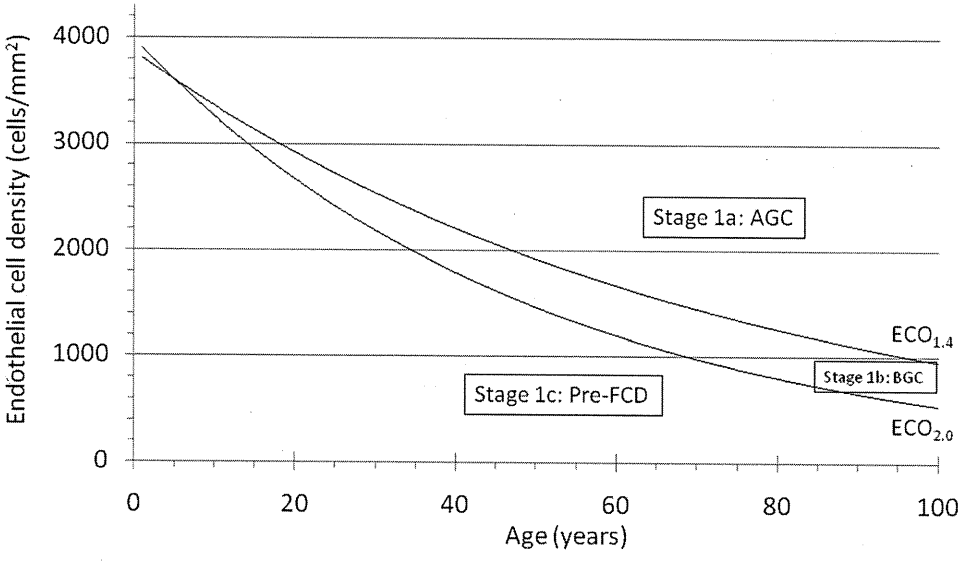


FIGURE 2. (A) The contour map of nonparametric density smoothing in stage 1. Stage 1 consisted of several peaks, and the age-ECD curve of 1.40% decrease rate ($ECO_{1.4}$, *green curve*) ran through a trough between peaks of high ECD group (*black asterisks*) and low ECD group (*red asterisks*). (B) High-density group in stage 1 above $ECO_{1.4}$. The age-ECD curve of this group (*red curve*) was close to that of stage 0 (*red dotted curve*), and the calculated decrease rate was 0.56%. (C) Low-density group in stage 1 below $ECO_{1.4}$. The age-ECD curve of this group (*red curve*) coincided with $ECO_{2.0}$ (*black dotted curve*), with a decrease rate of 2.00%.

spective, hospital-based review of total 1971 outpatients. In this study, we found a somewhat higher prevalence of guttata cornea than that found in previous reports in Japan. The prevalence of corneal guttae was reported to be 3.7% (1.5% in men, 5.5% in women) in Japan,[17,18] whereas it ranges from approximately 7% up to a remarkable 70.4% in North America, Iceland, and Europe.[1,8,19] In our study, the fact that subjects were hospital-based may have caused a higher prevalence. However, such bias does not have an effect on the validity of the mathematical model derived from the data. The following tendency of prevalence was apparent in our group of subjects: First, females were more predisposed to stages 1, 2, and 3 than were males, and the female ratio increased as stages progressed. Second, the prevalence of FCD was much smaller than stage 1. An increase in the female ratio in progressing stages suggested that sex may have some role not only in the onset but also the progression of the disease. Apparent difference of prevalence between FCD and stage 1 suggest the existence of a patient group in stage 1 that does not progress to corneal edema despite having guttata cornea.

Our model is based on the assumptions that the ECD at 5 years of age is common to all classes and that the decrease rate



Age (years)		20	30	40	50	60	70	80	90
ECD (cells/mm²)	$ECO_{1.4}$	2918	2537	2205	1917	1667	1449	1260	1095
	$ECO_{2.0}$	2667	2184	1788	1464	1198	981	803	658

FIGURE 3. Proposed classification of eyes in stage 1 based on $ECO_{1.4}$ and $ECO_{2.0}$. Eyes in stage 1a above $ECO_{1.4}$ were named AGC, which had a decrease rate as low as stage 0. Eyes in stage 1c below $ECO_{2.0}$ had a decrease rate as high as FCD (stages 2 and 3), and therefore, this stage was named pre-FCD. Stage 1b between $ECO_{1.4}$ and $ECO_{2.0}$ was named BGC. The table below the graph shows the coordinates of $ECO_{1.4}$ and $ECO_{2.0}$.

of ECD percentage per year) is constant but with a different value of each class. The use of these assumptions may be a debatable point when discussing the validity of our study. However, the results of our mathematical model show ECD decrease rates that are acceptable when compared with clinical observations. The decrease rate of 0.44% in stage 0 is within the range of values of normal unoperated eyes reported in the previous studies.[10,12–16] Furthermore, since $ECO_{1.4}$ and $ECO_{2.0}$ runs through a clearly defined trough between peaks on the scatterplot, and $ECO_{2.0}$ divided stages 0+1 and stages 2+3 or stage 1 and stages 2+3 with high sensitivity and specificity, we believe our mathematical model for classifying patients with guttae based on ECD decrease rates is adequate for predicting the prognosis.

The $ECO_{1.4}$ and $ECO_{2.0}$ curves based on our mathematical model divided stage 1 into three subgroups, stage 1a, 1b, and 1c. The ECD decrease rate of stage 1a was close to that of stage 0, that is, almost normal. Schnitzer and Krachmer reported on 44 relatives of 12 families with guttata cornea which appeared normal on slit-lamp examination and endothelial cell parameters.[20] These eyes presumably belonged to stage 1a of our classification. In addition, because the distribution of patients of stage 1a was located above $ECO_{1.4}$, the risk of progressing to corneal edema may be as low as stage 0. If a patient was on the curve of a 1.4% decrease rate, the ECD would be 1095 cells/mm^2 even when he was 90 years old. Presumption of low risk of stage 1 is supported by analysis of variance, showing that age-ECD curves of each stage had significant predictability.

It was surprising that the age-ECD curve of the low-density group of stage 1 (stages 1b and 1c) coincided completely with $ECO_{2.0}$. The former was calculated by the least-squares method of the low-density group of stage 1, whereas the latter was obtained from trough between peaks of stages 0 to 3 on scatterplots. This result suggests that the low-density group of stage 1 was located on the border between stage 0 and FCD. Eyes in stage 1c below $ECO_{2.0}$ have a decrease rate as high as FCD, suggesting that these eyes have a risk to progress to FCD, even if there was no corneal edema present. This was the rationale for referring to stage 1c as pre-FCD. Further prospective study of patients in stage 1b and 1c is needed to determine whether stage 1c is a preliminary stage of FCD.

Recently, several pathogenic mechanisms, such as oxidative stress or unfolded protein response, have been reported as causes of FCD.[21,22] The difference in resistance against such stress may cause the difference in decrease rates between stages. Previous reports suggested that ECD of some eyes with guttata cornea did not decrease significantly compared with normal eyes after cataract surgery,[7,23] whereas some eyes in other reports showed a significantly higher decrease.[24] When we adapted data from these reports to our classification, we found that most of the former eyes with no difference in ECD (18/21 eyes) were categorized as stage 1a, suggesting that our classification may be used to identify patients with a higher risk of endothelial damage due to external stress. Future studies on guttata corneas using our classification may help clarify the mechanism of FCD progression.

In conclusion, we assessed distribution and endothelial loss rate of guttata cornea stages 0 to 3 and determined new cutoff curves $ECO_{1.4}$ and $ECO_{2.0}$ by using scatterplots. Our mathematical model is a simple method for predicting the prognosis of patients with guttata cornea.

References

1. Weisenthal RW, Streeten BW, eds. *Posterior Membrane Dystrophies.* London: Elsevier Mosby; 2005.
2. Edelhauser HF, Ubels JL, eds. *The Cornea and the Sclera.* 10th ed. ed. St. Louis: Mosby; 2003.
3. Al-Yousuf N, Mavrikakis I, Mavrikakis E, Daya SM. Penetrating keratoplasty: indications over a 10 year period. *Br J Ophthalmol.* 2004;88(8):998-1001.
4. Dobbins KR, Price FW Jr, Whitson WE. Trends in the indications for penetrating keratoplasty in the midwestern United States. *Cornea.* 2000;19(6):813-816.
5. Kang PC, Klintworth GK, Kim T, et al. Trends in the indications for penetrating keratoplasty. 1980-2001. *Cornea.* 2005;24(7):801-803.
6. Krachmer JH, Purcell JJ Jr, Young CW, Bucher KD. Corneal endothelial dystrophy: a study of 64 families. *Arch Ophthalmol.* 1978; 96(11):2036-2039.
7. Kitagawa K, Fujisawa A, Mizuno T, Sasaki K. Twenty-three cases of primary cornea guttata. *Jpn J Ophthalmol.* 2001;45(1):93-98.
8. Adamis AP, Filatov V, Tripathi BJ, Tripathi RC. Fuchs' endothelial dystrophy of the cornea. *Surv Ophthalmol.* 1993;38(2):149-168.
9. Stocker FW. The endothelium of the cornea and its clinical implications. *Trans Am Ophthalmol Soc.* 1953;51:669-786.
10. Murphy C, Alvarado J, Juster R, Maglio M. Prenatal and postnatal cellularity of the human corneal endothelium: a quantitative histologic study. *Invest Ophthalmol Vis Sci.* 1984;25(3):312-322.
11. Nucci P, Brancato R, Mets MB, Shevell SK. Normal endothelial cell density range in childhood. *Arch Ophthalmol.* 1990;108(2):247-248.
12. Cheng H, Jacobs PM, McPherson K, Noble MJ. Precision of cell density estimates and endothelial cell loss with age. *Arch Ophthalmol.* 1985;103(10):1478-1481.
13. Ambrose VM, Walters RF, Batterbury M, Spalton DJ, McGill JI. Long-term endothelial cell loss and breakdown of the blood-aqueous barrier in cataract surgery. *J Cataract Refract Surg.* 1991; 17(5):622-627.
14. Numa A, Nakamura J, Takashima M, Kani K. Long-term corneal endothelial changes after intraocular lens implantation: anterior vs posterior chamber lenses. *Jpn J Ophthalmol.* 1993;37(1):78-87.
15. Bourne WM, Nelson LR, Hodge DO. Central corneal endothelial cell changes over a ten-year period. *Invest Ophthalmol Vis Sci.* 1997;38(3):779-782.
16. Rao SK, Ranjan Sen P, Fogla R, Gangadharan S, Padmanabhan P, Badrinath SS. Corneal endothelial cell density and morphology in normal Indian eyes. *Cornea.* 2000;19(6):820-823.
17. Kitagawa K, Kojima M, Sasaki H, et al. Prevalence of primary cornea guttata and morphology of corneal endothelium in aging Japanese and Singaporean subjects. *Ophthalmic Res.* 2002;34(3): 135-138.
18. Nagaki Y, Hayasaka S, Kitagawa K, Yamamoto S. Primary cornea guttata in Japanese patients with cataract: specular microscopic observations. *Jpn J Ophthalmol.* 1996;40(4):520-525.
19. Zoega GM, Fujisawa A, Sasaki H, et al. Prevalence and risk factors for cornea guttata in the Reykjavik Eye Study. *Ophthalmology.* 2006 Apr;113(4):565-569.
20. Schnitzer JI, Krachmer JH. A specular microscopic study of families with endothelial dystrophy. *Br J Ophthalmol.* 1981;65(6): 396-400.
21. Buddi R, Lin B, Atilano SR, Zorapapel NC, Kenney MC, Brown DJ. Evidence of oxidative stress in human corneal diseases. *J Histochem Cytochem.* 2002;50(3):341-351.
22. Engler C, Kelliher C, Spitze AR, Speck CL, Eberhart CG, Jun AS. Unfolded protein response in Fuchs endothelial corneal dystrophy: a unifying pathogenic pathway? *Am J Ophthalmol.* 2010;149(2):194-202-e2.
23. Stur M, Grabner G, Dorda W. Changes of the corneal endothelium following intracapsular cataract extraction with implantation of semiflexible anterior chamber lenses. I. Results of the early postoperative period. *Acta Ophthalmol (Copenh).* 1984;62(4):586-594.
24. Bourne WM, Nelson LR, Hodge DO. Continued endothelial cell loss ten years after lens implantation. *Ophthalmology.* 1994; 101(6):1014-1022.

Ocular Surface Reconstruction Using the Combination of Autologous Cultivated Oral Mucosal Epithelial Transplantation and Eyelid Surgery for Severe Ocular Surface Disease

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• PURPOSE: To assess the surgical combination of autologous cultivated oral mucosal epithelial transplantation and eyelid surgery used to treat patients with severe ocular surface disease and entropion.

• DESIGN: Observational case series.

• METHODS: Three patients with severe thermal and chemical injury were treated by the surgical combination of autologous cultivated oral mucosal epithelial transplantation and everting sutures to correct entropion. Their clinical outcomes and the efficacy of this surgical procedure were assessed.

• RESULTS: The ocular surfaces were successfully reconstructed with autologous cultivated oral mucosal epithelial sheets and everting sutures without any complications during the operations. In the course of a mean follow-up period of 30 months their clinical outcomes were assessed. Postoperative follow-up showed that the simultaneous everting sutures caused no problems with the cultivated oral mucosal epithelial sheet, and there were no severe complications such as infection or inflammation. During the follow-up period, in 2 of the 3 eyes the ocular surface and eyelid remained stable with no recurrence of entropion.

• CONCLUSION: This case series presents a surgical approach to treat severely scarred ocular surfaces using the combination of autologous cultivated oral mucosal epithelial transplantation and everting sutures. Clinical outcomes suggest that this combined surgical procedure is a safe and useful method for the treatment of patients with severe ocular surface disease and entropion. (Am J Ophthalmol 2011;152:195–201. © 2011 by Elsevier Inc. All rights reserved.)

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CORNEAL EPITHELIAL STEM CELL DEFICIENCY RESULTING from severe ocular surface disease (OSD) such as thermal and chemical injury, Stevens-Johnson syndrome, and ocular cicatricial pemphigoid leads to visual loss attributable to conjunctivalization, vascularization, opacification, and symblepharon.[1–3] Specifically, various degrees of pathologic symblepharon and entropion frequently occur in patients with severe OSD and disturb the stability of the ocular surface. Moreover, eyelid abnormalities can often make a severe corneal disease worse because eyelid margin rotation or structural abnormalities impair tear spreading and corneal wetting. The eyelid pathologies include keratinization and tarsal scarring that causes and aggravates limbal stem cell deficiency (LSCD), as first reported by Di Pascuale and associates.[4] These abnormalities were later graded by Sotozono and associates.[5] Malfunction of the fornix by symblepharon can cause ocular surface diseases such as dry eye, blindness-related microtrauma, and an inflamed ocular surface resulting from cicatricial entropion, mal-aligned lashes, and restriction of ocular motility. Therefore, it was recently reported that the selection of the proper surgical approach depends on the severity of pathogenic symblepharon.[6,7] Moreover, it was also recommended that the eyelid and fornix abnormalities be corrected prior to performing ocular surface reconstruction.[7–9] In these types of patients, it is necessary to reconstruct not only the ocular surface epithelium but also the formation of the eyelid.

To reconstruct the ocular surface in patients with severe OSD, a variety of surgical approaches such as keratoepithelioplasty, limbal transplantation, and amniotic membrane (AM) transplantation have been used.[10–17] Due to the recent progress that has been made with regenerative medicine techniques, there have now been studies reporting the use of cultivated epithelial transplantation.[18–20] Since 2002, we have performed 61 cases of autologous cultivated oral mucosal epithelial transplantation for patients with severe OSD.[21,22] Among those cases, we have experienced 3 cases of severe entropion in thermal and chemical injury. The treatment of those 3 cases required the surgical combination of autologous cultivated oral

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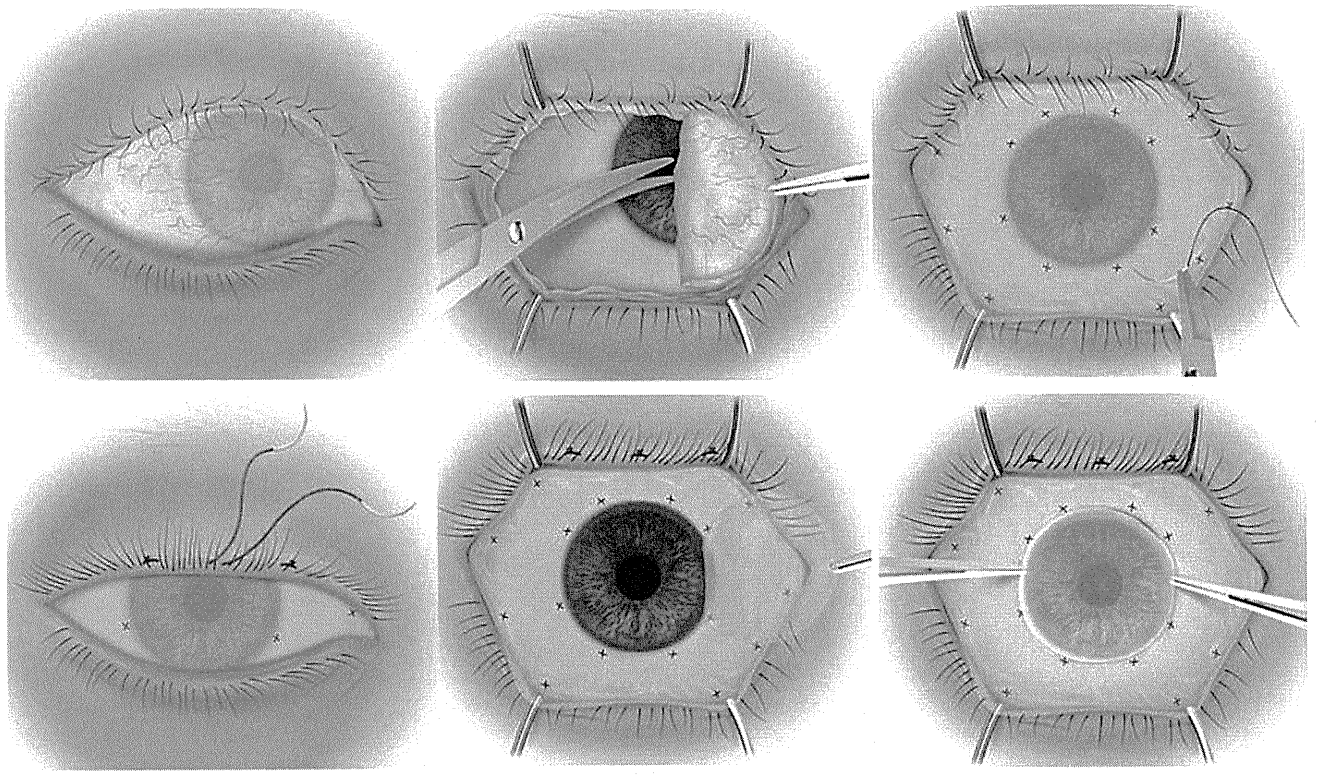

FIGURE 1. Surgical procedure used in the current study. (Top left) The eye manifested severe epithelial damage and entropion. (Top middle) The conjunctivalized tissue was completely removed by thin superficial keratectomy and peritectomy. (Top right) Transferred human amniotic membrane (AM) over the corneal surface. (Bottom left) A 6-0 nylon suture was passed through the eyelid from the tarsus side to the skin side. (Bottom middle) AM on the cornea was removed. (Bottom right) Finally, the autologous cultivated oral mucosal epithelial sheet was transferred onto the corneal surface.

mucosal epithelial transplantation and eyelid surgery, as we had experienced many previous cases where the ocular surface was severe damaged by cilia touching the surface because of entropion. In this present study, we report in detail for the first time the clinical outcome of using the surgical combination of autologous cultivated oral mucosal epithelial transplantation and eyelid surgery.

PATIENTS AND METHODS

THIS STUDY INVOLVED 3 EYES FROM 3 PATIENTS WITH total limbal deficiency that underwent autologous cultivated oral mucosal epithelial transplantation with everting sutures at Kyoto Prefectural University of Medicine, Kyoto, Japan, between August 2004 and October 2006. Their ages were 65 years (Case 1), 26 years (Case 2), and 32 years (Case 3). In Case 1, the primary reason for the patient's limbal deficiency was a severe, work-related chemical injury resulting from his right eye being exposed to the alkali of an aqueous-fluid solvent used for cleaning metal. He was found to have corneal edema and the manifested persistent epithelial defect (PED) involved disappearance of the palisades of Vogt and strong conjunctival hyperemia and chemosis with lower-eyelid entropion and symblepharon. The grading score for symblepharon severity was grade Ia 3+ (Kheirkhah score) and grade 2 (Sotozono score) according to the previous reports.[5,7] In Case 2, the primary reason for the patient's limbal deficiency was a severe thermal injury to his left eye attributable to its being hit by a bottle-rocket firework. He was found to have corneal opacity and the total corneal epithelial defect involved the disappearance of the palisades of Vogt with a large amount of hypopyon, conjunctivalization, entropion, symblepharon, and fibrovascular cicatrix to the upper fornix with the appearance of severe necrotic change in the conjunctiva and eyelid. The grading score for symblepharon severity was grade Ia 2+ (Kheirkhah score) and grade 1 (Sotozono score) according to the previous reports.[5,7] In Case 3, the primary reason for the patient's limbal deficiency was a severe thermal injury to his left eye attributable to its being hit by a bottle-rocket firework. He was found to have manifested almost-total PED involving the disappearance of the palisades of Vogt with conjunctivalization, entropion, and symblepharon, and the appearance of a severely scarred upper eyelid that involved fibrosis to the upper fornix. The grading score for symblepharon severity was grade Ia 2+ (Kheirkhah score) and grade 2 (Sotozono score) according to the previous reports.[5,7]

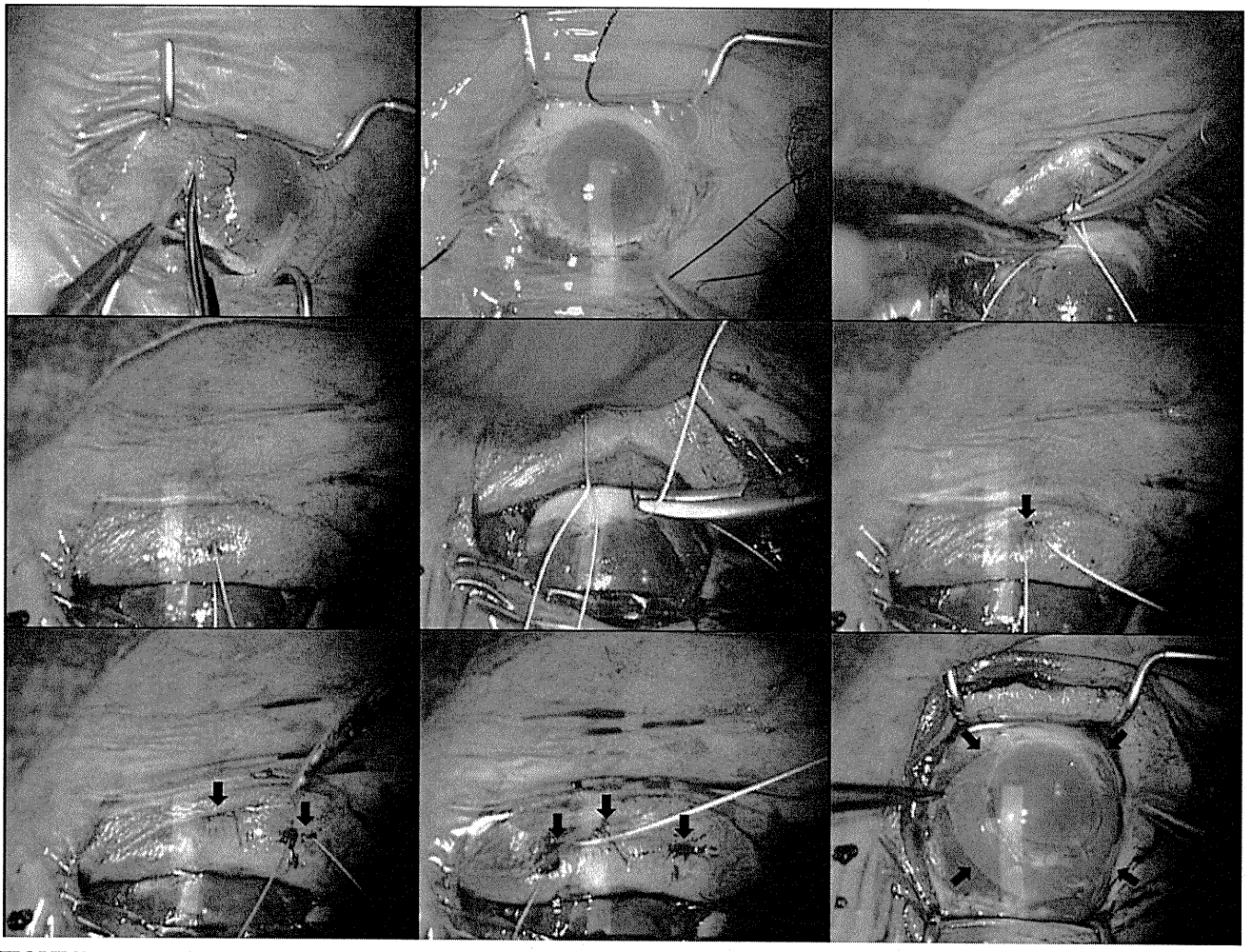

FIGURE 2. Surgical appearance of autologous cultivated oral mucosal epithelial transplantation and everting sutures in Case 1. (Top left) We completely removed the conjunctivalized tissue by thin superficial keratectomy and peritectomy. (Top center) Transferred human amniotic membrane (AM) was placed over the corneal surface. (Top right, Middle left) First, a 6-0 nylon suture was passed through the eyelid from the tarsus side to the skin side. (Middle center) The second needle of that same suture was then passed through in the same manner. (Middle right) Those 2 sutures were then tied off. (Bottom left and Bottom center) This procedure was repeated on both sides. (Bottom right) Finally, the autologous cultivated oral mucosal epithelial sheet was transferred onto the corneal surface.

All 3 patients were diagnosed as stem cell deficient on the basis of the disappearance of the palisades of Vogt. All 3 eyes manifested severe epithelial damage and entropion, and PED was induced in all 3 eyes because of cilia touching the ocular surface.

In this study, cultivated oral mucosal epithelial sheets were generated using the procedure previously reported.[21–23] Briefly, the presence of healthy oral mucosa in each patient was confirmed by a dentist. Human amniotic membrane was harvested at the time of caesarean section. Autologous oral epithelial cells, grown for 2 weeks on denuded AM and co-cultured with mitomycin C–inactivated 3T3 fibroblasts, were airlifted for 1 to 2 days.

The surgical procedure used in this study is illustrated in Figure 1.[21–23] Briefly, the conjunctivalized tissue of each patient was completely removed by performing a thin superficial keratectomy and peritectomy (Figures 1 and 2). Subconjunctival spaces were then treated with 0.04% mitomycin C for 5 minutes, followed by human AM being transplanted over the ocular surface and then sutured to the sclera. All 3 cases required an additional surgical procedure to address existing entropion of either the upper or lower eyelid. For this procedure, we selected everting sutures to connect the skin and tarsus when surgically reconstructing the abnormal eyelid with the existing destroyed palpebra structure that resulted from chemical or thermal injury. First, a 6-0 nylon suture was passed through the eyelid from the tarsus side to the skin side (Figures 1 and 2), followed by the second needle of that same suture being passed through in the same manner (Figures 1 and 2). The 2 ends of that suture were then tied off and implanted under the skin. This was repeated 2 or 3 times at the appropriate positions in relation to the first suture, with the number of additional sutures needed being determined by the severity of the entropion of each particular case

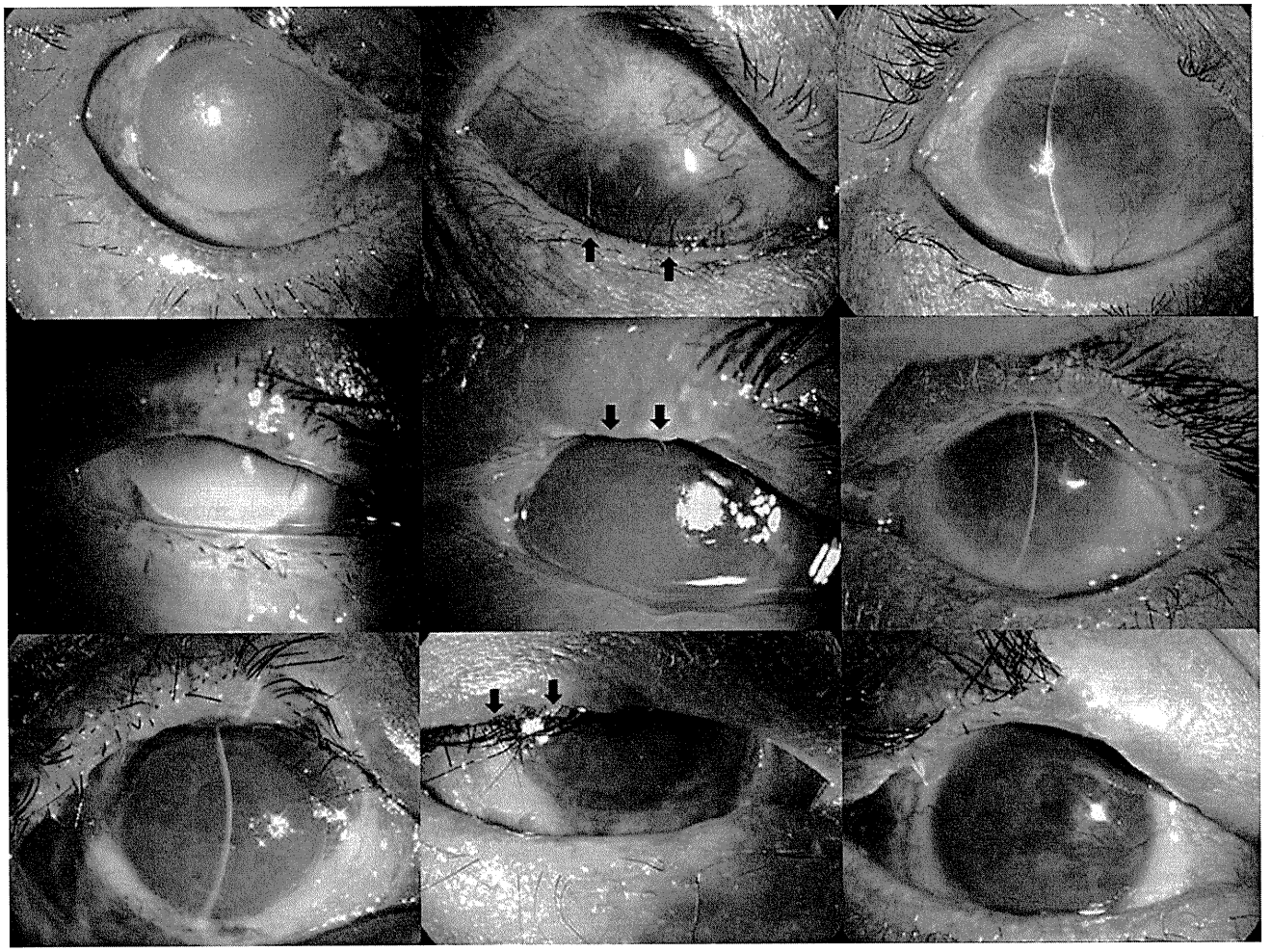

FIGURE 3. Clinical appearance before and after reconstruction using autologous cultivated oral mucosal epithelial transplantation and everting sutures for severe ocular surface disease. (Top left) Case 1 with alkali injury soon after arrival at our hospital. (Top center) Preoperatively, there was total conjunctivalization with symblepharon and lower-eyelid entropion. (Top right) Seven months after surgery, the cultivated oral mucosal epithelial sheet showed no epithelial defect and the ocular surface was stable. (Middle left) Case 2 with a fireworks-induced thermal injury soon after arrival at our hospital, manifesting severe epithelial damage with hypopyon, corneal opacity, ischemic conjunctival edema, and symblepharon. (Middle center) Preoperatively, there was upper-eyelid entropion with a severe scar and cilia had touched the corneal surface. (Middle right) After surgery, the cultivated oral mucosal epithelial sheet was stable with no recurrence of entropion. (Bottom left) Case 3 with a fireworks-induced thermal injury soon after arrival at our hospital, manifesting total corneal epithelial defect. (Bottom center) Preoperatively, there was persistent epithelial defect (PED), symblepharon, and scarred entropion of the upper eyelid. (Bottom right) Six weeks after surgery, the entropion relapsed and symblepharon also gradually occurred.

(Figures 1 and 2). Finally, the cultivated autologous oral mucosal epithelial sheet was transferred onto the corneal surface and then sutured onto the surface with 10-0 nylon (Figures 1 and 2). The ocular surface was then protected with a medical-use bandage contact lens. The 3 patients were followed up for 11, 29, and 50 months, respectively (mean follow-up period: 30 months).

CLINICAL RESULTS

IN ALL 3 CASES, WE WERE ABLE TO PERFORM THE OCULAR surface reconstruction using the combination of autologous cultivated oral mucosal epithelial transplantation and everting sutures without any complications during the operations. Postoperative follow-up showed that the simultaneous eyelid surgery caused no problems with the transplanted cultivated oral mucosal epithelial sheet. In addition, severe complications such as infection and inflammation were not observed in relation to the everting sutures. During the follow-up period, in 2 of the 3 eyes (Cases 1 and 2) the ocular surface and eyelid remained stable with no recurrence of entropion; however, in 1 eye (Case 3) there was a recurrence of entropion that induced an epithelial defect.

• CASE 1: Case 1 involved a 65-year-old man in the acute phase of alkali injury (Kinoshita grading score IIIb accord-

ing to the previously reported classification[24]) with severe corneal stromal opacity in July 2004. The patient was diagnosed as stem cell deficient on the basis of the disappearance of the palisades of Vogt and the manifested PED with marginal lower-eyelid entropion and symblepharon (grading Ia3+[7] and grading 2[5] according to the previously reported classifications) (Figure 3, Top left). The PED was caused by cilia touching the ocular surface (Figure 3, Top center). Preoperative visual acuity was 12/200, and autologous cultivated oral mucosal epithelial transplantation combined with everting sutures for the lower-eyelid entropion was performed. Postoperatively, the patient's ocular surface was stable with no recurrence of entropion (Figure 3, Top right). However, this case required penetrating keratoplasty 11 months after surgery because of the pre-existing corneal stromal opacity. The surviving oral mucosal epithelium then resulted in the formation of a stable ocular surface.

• CASE 2: Case 2 involved a 26-year-old man in the acute phase of thermal injury (Kinoshita grading score IIIb[24]) resulting from exposure to fireworks. The patient was diagnosed as stem cell deficient on the basis of disappearance of the palisades of Vogt and the manifested severe epithelial damage with hypopyon, conjunctivalization, entropion, and symblepharon (grading Ia2+[7] and grading 1[5] according to the previously reported classifications) (Figure 3, Middle left). Specifically, the patient's upper eyelid was entropion with a severe scar (Figure 3, Middle center). Autologous cultivated oral mucosal epithelial transplantation combined with everting sutures for the upper-eyelid entropion was performed. Postoperatively, after 29.1 months of follow-up, the patient's ocular surface was stable with no recurrence of entropion and the structure of the eyelid was maintained (Figure 3, Middle right).

• CASE 3: Case 3 involved a 32-year-old man in the acute phase of thermal injury (Kinoshita grading score IIIb[24]) resulting from exposure to fireworks. The patient was diagnosed as stem cell deficient on the basis of the disappearance of the palisades of Vogt and the manifested PED with conjunctivalization, entropion, and symblepharon (grading Ia2+[7] and grading 1[5] according to the previously reported classifications) (Figure 3, Bottom left). Specifically, the upper eyelid was entropion with a severe scar (Figure 3, Bottom center). Autologous cultivated oral mucosal epithelial transplantation combined with everting sutures for the upper-eyelid entropion was performed. Although a small epithelial defect occurred postoperatively, it eventually healed with the clinical use of a bandage contact lens. For this patient, the everting sutures were effective for a short period of time (Figure 3, Bottom right). However, after 6 weeks postoperatively, there was a recurrence of the entropion and a gradual recurrence of the symblepharon. Moreover, an epithelial defect appeared resulting from cilia touching the ocular surface. Though the ocular surface had been stable because of the clinical-use contact lens, after 15 months of follow-up, conjunctival reconstruction by autologous cultivated oral mucosal epithelial transplantation combined with upper-eyelid splitting was performed. After 50.7 months of follow-up, the surviving oral mucosal epithelium formed a stable ocular surface.

DISCUSSION

THIS STUDY PRESENTS A SIMULTANEOUS SURGICAL APproach to treat patients with severe limbal deficiency disorder. This surgical approach consists of a combination of autologous cultivated oral mucosal epithelial transplantation and everting sutures performed during 1 operation. The results of this study showed that everting sutures are safe to perform in combination with a cultivated epithelial sheet being transplanted onto the ocular surface. The 3 patients in this study were followed up for a mean period of 30 months, and our findings showed that their respective ocular surfaces and eyelid structures were successfully reconstructed by use of this combined surgical approach.

In general, both mild and moderate symblepharon can be corrected by AM transplantation alone when the remaining conjunctiva in the symblepharon is moved from the bulbar region to the tarsal region. However, all 3 patients presented in this study were diagnosed with total corneal epithelial stem cell deficiency and had manifested severe epithelial damage, symblepharon, and entropion. Therefore, the patients required reconstruction of not only the eyelid and conjunctival surface but also the corneal surface, by supplying it with epithelial stem cells. For this reason, we reconstructed the corneal surface by the transplantation of a cultivated oral mucosal epithelial sheet and reconstructed the conjunctival surface by the transplantation of human AM. The advantage of performing cultivated oral mucosal epithelial transplantation is that the transplanted epithelial sheet contains stem cells that help to reconstruct the corneal surface and maintain the ocular surface integrity.

This study involved 3 cases of burn injury that caused cicatricial entropion. Entropion is caused by a relative shortage of posterior lamella compared with the anterior lamella attributable to the cicatricial change in the tarsal-conjunctival layer. Conjunctival scarring by trauma or inflammation causes shortening of the fornices. There have been several reported approaches used for the reconstruction of the eyelid in patients with these types of severe OSD. However, the optimal surgical procedure is still an open question. Kemp and Collin[25] demonstrated that minimal and moderate entropion, such as minimal conjunctivalization of the eyelid margin, cilia touching the ocular surface, and thickening of the tarsal plate, can be treated by anterior lamellar repositioning and splitting the

eyelid margin. A case of severe eyelid disturbance requires rotation of the distal tarsal conjunctiva, such as a lamellar division. Previous reports have shown that if the tarsal-conjunctival layer does not advance enough by shortening of the conjunctival fornix, a hard-palate mucosal graft can be inserted between the 2 cut edges of the tarsus or as a spacer into the posterior eyelid lamella.[26–29] In this present study, these types of eyelid surgeries were not used, simply because they are more invasive to the damaged eyelid and strongly induce postoperative inflammation. Surgical methods for treating entropion such as the Jones method and the Hotz method are well known, but these methods are also surgically more invasive procedures than everting sutures. Moreover, everting sutures have less incidence of inflammation. Therefore, we considered the everting sutures safe to use in conjunction with the cultivated epithelial sheet on the ocular surface. However, since the everting sutures can result in an increased rate of recurrence, we explain this risk in detail to the patients and their immediate family prior to the operation.

Case 3 in this study unfortunately experienced postoperative recurrence of entropion and symblepharon. For this patient we were able to reconstruct the ocular surface with a second operation that involved eyelid splitting and autologous cultivated oral mucosal epithelial transplantation for the reconstruction of conjunctiva fornix. We now understand the importance of selecting the appropriate surgical procedure depending on the severity of the entropion and palpebral disorder, as we have learned that simple everting sutures are sometimes insufficient for eyelid reconstruction in the most severe cases.

The results of this study demonstrate that the combined surgical procedure of autologous cultivated oral mucosal epithelial transplantation and everting sutures is a useful approach for the treatment of severe ocular surface disorders with associated eyelid abnormality. Further study is required to develop new methods for eyelid surgery that lower the risk of complications following ocular surface reconstruction.

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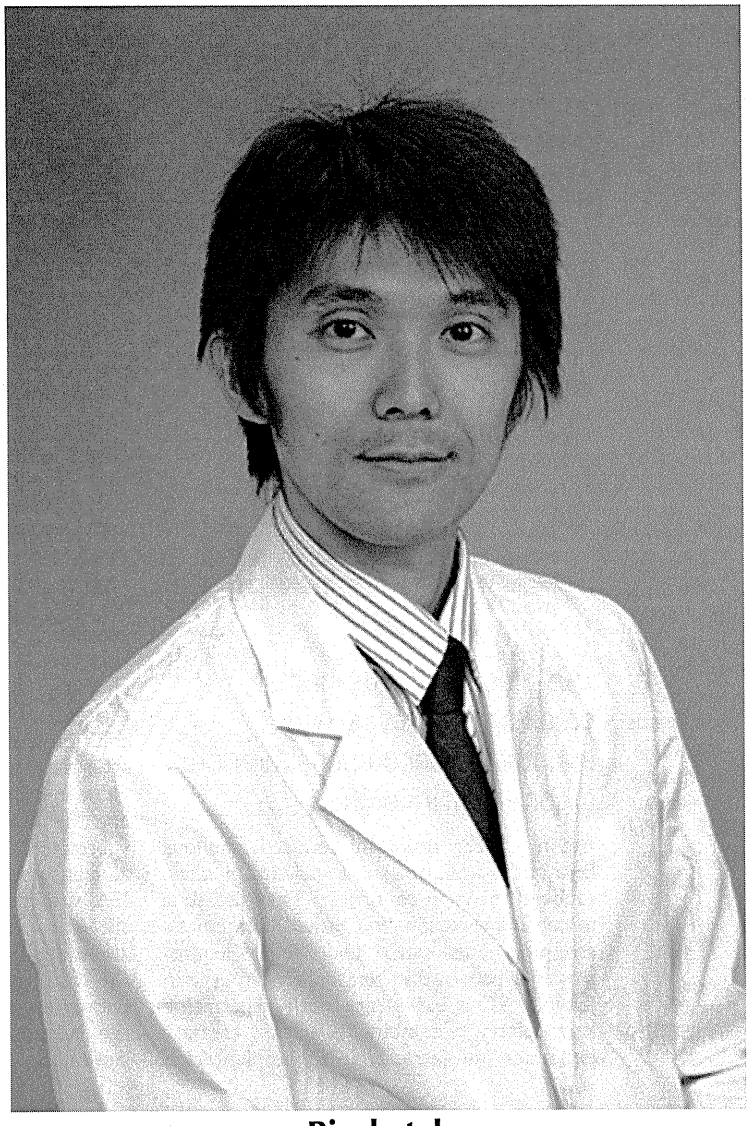
REFERENCES

1. Shapiro MS, Friend J, Thoft RA. Corneal re-epithelialization from the conjunctiva. Invest Ophthalmol Vis Sci 1981; 21(1):135–142.
2. Dua HS, Forrester JV. The corneoscleral limbus in human corneal epithelial wound healing. Am J Ophthalmol 1990; 110(6):646–656.
3. Tsai RJF, Sun TT, Tseng SCG. Comparison of limbal and conjunctival autograft transplantation in corneal surface reconstruction in rabbits. Ophthalmology 1990;97(4):446–455.
4. Di Pascuale MA, Espana EM, Liu DT, et al. Correlation of corneal complications with eyelid cicatricial pathologies in patients with Stevens-Johnson syndrome and toxic epidermal necrolysis syndrome. Ophthalmology 2005;112(5):904–912.
5. Sotozono C, Ang LP, Koizumi N, et al. New grading system for the evaluation of chronic ocular manifestations in patients with Stevens-Johnson syndrome. Ophthalmology 2007;114(7):1294–1302.
6. Tseng SCG, Di Pascuale MA, Liu DT, Gao YY, Baradaran-Rafii A. Intraoperative mitomycin C and amniotic membrane transplantation for fornix reconstruction in severe cicatricial ocular surface diseases. Ophthalmology 2005; 112(5):896–903.
7. Kheirkhah A, Blanco G, Casas V, Hayashida Y, Raju VK, Tseng SC. Surgical strategies for fornix reconstruction based on symblepharon severity. Am J Ophthalmol 2008;146(2):266–275.
8. Liang L, Sheha H, Tseng SC. Long-term outcomes of keratolimbal allograft for total limbal stem cell deficiency using combined immunosuppressive agents and correction of ocular surface deficits. Arch Ophthalmol 2009;127(11): 1428–1434.
9. deSousa JL, Daya S, Malhotra R. Adnexal surgery in patients undergoing ocular surface stem cell transplantation. Ophthalmology 2009;116(2):235–242.
10. Thoft RA. Keratoepithelioplasty. Am J Ophthalmol 1984; 97(1):1–6.
11. Kenyon KR, Tseng SCG. Limbal autograft transplantation for corneal surface disorders. Ophthalmology 1989;96(5): 709–723.
12. Kinoshita S, Ohashi Y, Ohji M, et al. Long-term results of keratoepithelioplasty in Mooren's ulcer. Ophthalmology 1991;98(4):438–445.
13. Tsai RJ, Tseng SC. Human allograft limbal transplantation for corneal surface reconstruction. Cornea 1994;13(5):389–400.
14. Kim JC, Tseng SC. Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. Cornea 1995;14(5):473–484.
15. Tsubota K, Satake Y, Ohyama M, et al. Surgical reconstruction of the ocular surface in advanced ocular cicatricial

pemphigoid and Stevens-Johnson syndrome. Am J Ophthalmol 1996;122(1):38–52.
16. Tsubota K, Satake Y, Kaido M, et al. Treatment of severe ocular surface disorders with corneal epithelial stem-cell transplantation. N Engl J Med 1999;340(22):1697–1703.
17. 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(7):1285–1290.
18. Pellegrini G, Traverso CE, Franzi AT, Zingirian M, Cancedda R, DeLuca M. Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. Lancet 1997;349(9057):990–993.
19. 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.
20. Koizumi N, Inatomi T, Suzuki T, Sotozono C, Kinoshita S. Cultivated corneal epithelial stem cell transplantation in ocular surface disorders. Ophthalmology 2001;108(9):1569–1574.
21. Nakamura T, Inatomi T, Sotozono C, Amemiya T, Kanamura N, Kinoshita S. Transplantation of cultivated autologous oral mucosal epithelial cells in patients with severe ocular surface disorders. Br J Ophthalmol 2004;88(10):1280–1284.
22. Inatomi T, Nakamura T, Koizumi N, Sotozono C, Yokoi N, Kinoshita S. Midterm results on ocular surface reconstruction using cultivated autologous oral mucosal epithelial transplantation. Am J Ophthalmol 2006;141(2):267–275.
23. Inatomi T, Nakamura T, Kojyo M, Koizumi N, Sotozono C, Kinoshita S. Ocular surface reconstruction with combination of cultivated autologous oral mucosal epithelial transplantation and penetrating keratoplasty. Am J Ophthalmol 2006;142(5):757–764.
24. Kinoshita S, Manabe R. Chemical burns. In: Brightbill FS, ed. Corneal Surgery. St Louis: Mosby; 1986:370–379.
25. Kemp EG, Collin JR. Surgical management of upper lid entropion. Br J Ophthalmol 1986;70(8):575–579.
26. Callahan A. Correction of entropion from Stevens-Johnson syndrome: use of nasal septum and mucosa for severely cicatrized eyelid entropion. Arch Ophthalmol 1976;94(7):1154–1155.
27. McCord CD, Chen WP. Tarsal polishing and mucous membrane grafting for cicatricial entropion, trichiasis and epidermalization. Ophthalmic Surg 1983;14(12):1021–1025.
28. Mannor GE, Mathers WD, Wolfley DE, Martinez JA. Hard-palate mucosa graft in Stevens-Johnson syndrome. Am J Ophthalmol 1994;118(6):786–791.
29. Goldberg RA, Joshi AR, McCann JD, Shorr N. Management of severe cicatricial entropion using shared mucosal grafts. Arch Ophthalmol 1999;117(9):1255–1259.



Biosketch

Kazunori Takeda, MD, is a clinical doctor of the Department of Ophthalmology at Kyoto Prefectural University of Medicine, Kyoto, Japan, where he received clinical training. He then spent 2 years as a staff surgeon at the Department of Ophthalmology, Maizuru Red Cross Hospital. Dr. Takeda's current interests include the treatment of ocular surface disorders through the use of regenerative medicine procedures.

20 prism dioptre (pd). To our best knowledge, there is little empirical data indicating the degree of sensitivity to magnitude of misalignment as a function of age. As such, claiming that children can detect a magnitude of 20 pd is an unsubstantiated assumption.

We would also like to clarify that the images given to our participants were not solely presented as pairs of the same child with and without conspicuous exotropia. The images shown have paired orthotropic and exotropic images of different children. Hence, the methodology, with respect to image presentation, is similar to that of Anderson *et al*.

We believe that our study design was appropriate for the purpose of investigating the effect of obvious exotropia on young children's social reactions. While we agree that many factors other than physical appearance contribute to playmate choice, first impression including a noticeable physical deviation from what is normal might reduce the willingness of a child to engage in future social interactions with a potential playmate. Our study clearly demonstrates that children as young as 5 years old are already capable of identifying and reacting negatively towards images of peers with obvious exotropia. This finding warrants the need to employ appropriate measures in educating parents regarding the possible *preventable* psychosocial impact associated with strabismus, particularly for children with obvious exotropia.

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REFERENCES

1. **Lukman H,** Kiat JE, Ganesan A, *et al*. Strabismus-related prejudice in 5–6-year-old children. *Br J Ophthalmol* 2010;**94**:1348–51.
2. **Johns HA,** Manny RE, Fern KD, *et al*. The effect of strabismus on a young child's selection of a playmate. *Ophthal Physiol Opt* 2005;**25**:400–7.
3. **Lukman H,** Kiat JE, Ganesan A, *et al*. Negative social reaction to strabismus in school children aged 8–12 years. *JAAPOS*. In Press.

The blood-aqueous barrier breakdown in eyes with endothelial decompensation after argon laser iridotomy

Argon laser iridotomy-induced bullous keratopathy (ALI-BK) is a growing medical problem in Asian countries.[1] This disease is a common reason for either Descemet's stripping automated endothelial keratoplasty or penetrating keratoplasty surgery in Japan.[2 3] Our experience with penetrating keratoplasty, combined with cataract extraction and intraocular lens implantation in eyes with ALI-BK, is typically characterised by an aggressive anterior-chamber inflammatory response, both intraoperatively and postoperatively, as compared with pseudophakic and aphakic BK, corneal opacity, and corneal scarring. Therefore, we speculate that in eyes with ALI-BK, the disease tends to weaken the blood-aqueous barrier. We quantitatively evaluated the blood-aqueous barrier in eyes with early endothelial decompensation following ALI using iris fluorescein angiography (IFA) and fluorophotometry.

SUBJECTS AND METHODS

This study involved nine eyes of seven patients with early endothelial decompensation following ALI, as well as eight eyes of four normal volunteers as a control. Relevant ethical committees approved the study protocol. A slit-lamp anterior fluorophotometer (FL-500; Kowa Company, Nagoya, Japan) was used to measure the blood-aqueous barrier function.[4] After the autofluorescence values in the anterior chamber were measured, an intravenous injection of 10 ml of 10% sodium fluorescein solution was administered. At 10 and 30 min after the injection, the fluorescence intensity value in the anterior chamber was measured, and the value of autofluorescence was then subtracted. From this value, the blood-aqueous barrier function was evaluated. IFA was performed with a photographic slit-lamp (SL-7F; Topcon, Tokyo, Japan) according to the published method.[5] After the intravenous injection of fluorescein, photographs were taken at 10 s intervals at the early phase and at 60 s intervals at the late phase.

Table 1 Clinical characteristics and outcome of fluorescein intensity values in eyes with endothelial decompensation after ALI and in normal controls

	Case no.	Age (years)	Eye	Purpose for ALI	Cornea	Region of the corneal oedema	ECD	Fluorescein intensity values (photon counts/s)	
								10 min	30 min
Patients with endothelial decompensation after ALI	1	63	L	Prophylactic	Early endothelial decompensation	–	309	202.0	435.0
	2	77	R	Prophylactic	Early endothelial decompensation	–	704	129.5	185.9
			L	Prophylactic	Early endothelial decompensation	Lower	415	381.4	428.2
	3	60	R	Prophylactic	Early endothelial decompensation	Upper	391	258.2	499.5
	4	75	L	Prophylactic	Early endothelial decompensation	Upper	525	977.6	1060.1
	5	73	R	Acute attack	Endothelial decompensation	Upper	–	648.5	1333.3
			L	Acute attack	Endothelial decompensation	Upper	–	1489.2	1665.8
	6	76	L	Acute attack	Early endothelial decompensation, band keratopathy	–	377	1227.7	2339.3
	7	82	R	Acute attack	Early endothelial decompensation	Lower	425	564.6	728.8
	Mean±SD	72.3±7.9						653.2±480.9	964.0±704.7
Normal control eyes	1	69	R	–	Clear	–	3472	26.1	105.1
			L	–	Clear	–	2941	12.6	92.7
	2	69	R	–	Clear	–	2976	11.9	101.2
			L	–	Clear	–	3215	13.9	96.0
	3	72	R	–	Clear	–	2444	22.5	58.5
			L	–	Clear	–	2375	39.2	72.5
	4	70	R	–	Clear	–	3012	12.5	74.3
			L	–	Clear	–	2840	19.7	65.3
	Mean±SD	70.0±1.4						19.8±9.5	83.2±17.6

Values are presented as the mean±SD in the bottom line.
ALI, argon laser iridotomy; ECD, endothelial cell density (cells/mm^2); L, left; R, right.

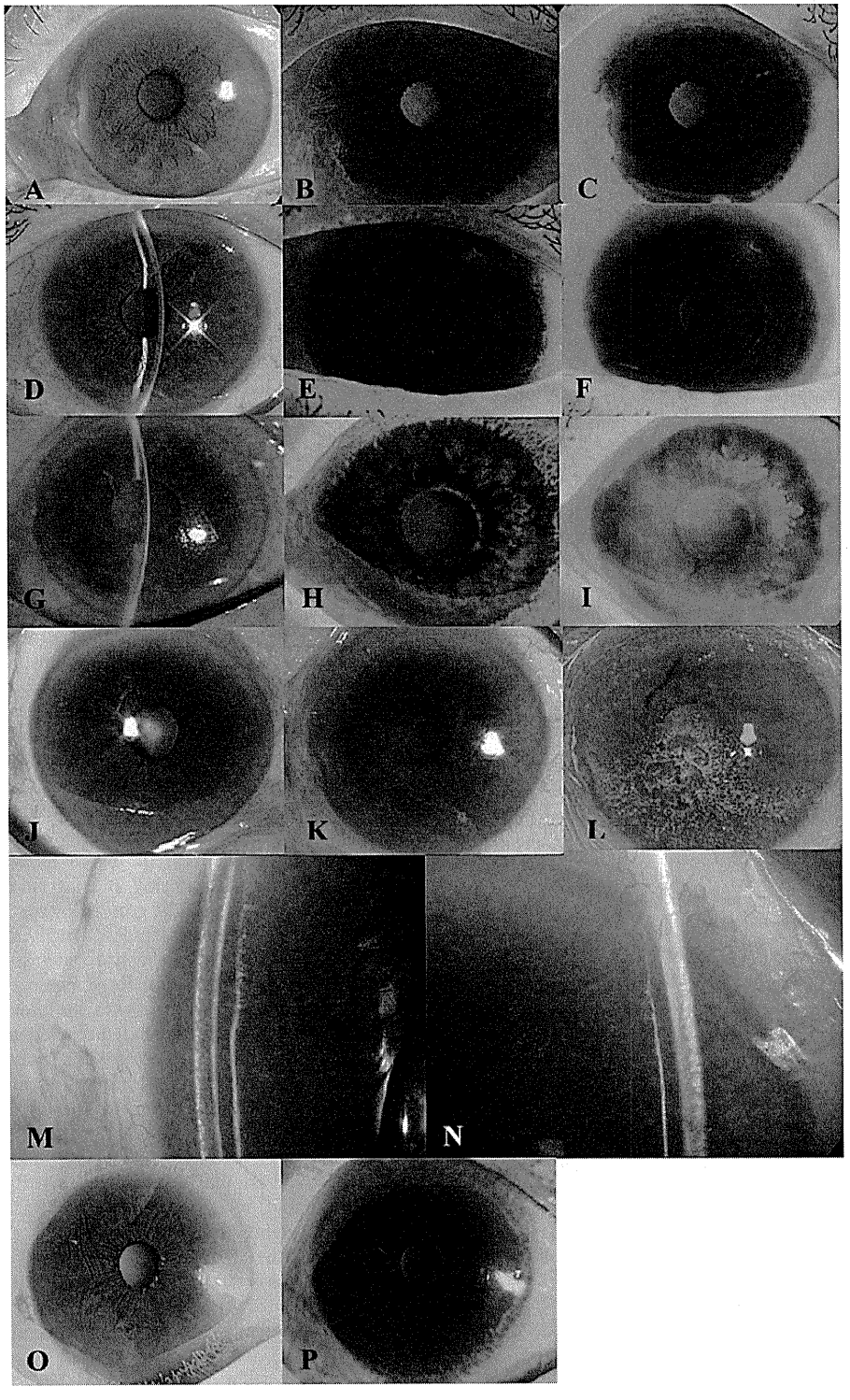

Figure 1 Clinical appearance in a normal control eye (A–C) and in three representative cases with early endothelial decompensation after argon laser iridotomy (ALI) (D–F, G–I, J–P). Iris fluorescein angiography (IFA) in the early phase (B, E, H, O) and in the late phase (C, F, I, P). Slit-lamp biomicroscopy shows a clear cornea in the normal control eye (A). IFA shows no dye leakage at both the early and late phases in the control eye (B, C). A clear cornea was seen in the left eye of a 63-year-old male patient (Case 1); anterior chamber depth is shallow and a large iridotomy is visible in the 1 o'clock position of the iris (D). IFA sequences of Case 1 (E, F): the dye leakage is seen at the ALI site and the spot leakage is also seen in the pupillary margin in the early phase (E). The dye leakages gradually increase in the late phase (F). Corneal oedema present in the inferior segment of the right eye of an 82-year-old female patient (Case 7); slit-lamp biomicroscopy shows a shallow anterior chamber and ALI is visible at the 10 o'clock position of the iris (G). IFA sequences of Case 7 (H, I); the dye leakage can be seen covering a wide area of the iris as well as at the pupillary margin at the early phase (H). The leaking gradually increases, persisting in the late phases (I). Clinical appearance of an eye with ALI-induced endothelial decompensation in a 77-year-old female patient (Case 2) (J–P). The cornea is clear and ALI is visible at the 10 o'clock position of the iris in the right eye (J). The cornea of the left eye is hazy due to corneal oedema and ALI is visible at the 2 o'clock position of the iris (K). Fluorescein staining demonstrating an irregular staining pattern over the localised area of oedema in the left eye (L). Note that the ALI window was not opened by only the ALI-produced atrophic scar (M, N). IFA sequences in the right eye (O, P); the dye leakage spots are seen in the pupillary margin; on the other hand, slight leakage is only seen at the ALI site in the early phase (O). The dye leakages gradually increase in the late phase (P).

RESULTS

The fluorescein intensity values and clinical characteristics of the patients and normal controls are listed in table 1. The fluorescein intensity values in the anterior chamber in eyes with endothelial decompensation after ALI, both at 10 and 30 min after the injection, were significantly higher than those obtained from the normal control eyes (p=0.0004). Patients with a history of acute glaucoma attack had significantly higher fluorescein intensity values compared with patients with prophylactic ALI (at 10 min, p=0.0300; at 30 min, p=0.0098). In all subjects, IFA revealed dye leakage in the pupillary margin and the ALI site, which became more intense in the late phases (figure 1D–F). Especially in cases with a history of acute glaucoma attack, the dye leakage was extensive; the dye spread was patchy and irregular in size and shape in the early phases, and the dye remained bright for a long period of time (figure 1G–I). In our study, a case in which only the ALI-produced atrophic scar remained in the iris without an ALI window was included (Case 2, figure 1J–P). Surprisingly, although the ALI window was not opened, corneal oedema occurred at the lower part of the cornea (figure 1K,L). IFA also revealed spots of dye leakage in the pupillary margin; however, only a slight leakage was observed at the ALI site (figure 1O,P). In the normal control

group, IFA revealed no dye leakage from the early to late phases (figure 1A–C).

COMMENT

Our results indicate that eyes with endothelial decompensation after ALI may actually have a chronic postsurgical breakdown of the blood-aqueous barrier. Presumably, the predisposition to postoperative inflammatory reactions in patients with ALI-BK appears to be caused by the manifested impairment of the blood-aqueous barrier. Although the reason why such a subclinical change may continue for a long period of time after ALI is unclear, the post-ALI endothelial decompensation may possibly be due to the humoral transport of substances such as prostaglandins and cytokines (eg, TGF-β1) in the anterior segment.

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REFERENCES

1. **Ang LP,** Higashihara H, Sotozono C, *et al*. Argon laser iridotomy-induced bullous keratopathy-a growing problem in Japan. *Br J Ophthalmol* 2007;**91**:1613–15.
2. **Shimazaki J,** Amano S, Uno T, *et al*. National survey on bullous keratopathy in Japan. *Cornea* 2007;**26**:274–8.
3. **Shimazaki J,** Uchino Y, Tsubota K. Late irreversible corneal oedema after laser iridotomy. *Br J Ophthalmol* 2009;**93**:125–6.
4. **Yokoi N,** Kinoshita S. Clinical evaluation of corneal epithelial barrier function with the slit-lamp fluorophotometer. *Cornea* 1995;**4**:485–9.
5. **Brancato R,** Bandello F, Lattanzio R. Iris fluorescein angiography in clinical practice. *Surv Ophthalmol* 1997;**42**:41–70.

Changing the status quo bias

We read with interest the article by Durnian and Clark who presented a retrospective cohort of infants that fell outside evidence level B guidelines for screening retinopathy of prematurity (ROP) and we wish to discuss some further points.[1] The data presented in table 1 highlight that all 11 babies would be missed if screening was according to level B evidence and 2 of these would still be missed using the good practice points evidence. An alternative conclusion would be that if the guidelines had been amended to screen infants under 32 weeks or 1251 g then one extra baby would have been missed (who fortunately did not require treatment). It is also debatable based on the information presented, if any of the babies absolutely required treatment as others may have opted to observe the fibrovascular ridge seen in zone 3 in cases 3 and 12. The screening criteria should allow the test to be not only highly sensitive but also specific. In order to present a complete discussion, we would ideally like to know over the same time period how many examinations would have been avoided if the screening criteria were at the secondary level (B) and also importantly if the criteria were amended to less than 32 weeks or 1251 g.

The article also stimulates thought as to what currently defines the population at risk of developing ROP. We have moved from seeing more mature babies with ROP with higher average birth weights to current times where the survival of very premature infants is higher and consequently ROP is seen in a population that has extremely low birth weights, at least in highly developed countries. The inclusion of the Danish cohort from 1982 to 1987 in the Royal College Guidelines on ROP 2008 was offered in part as evidence for the current criteria, but these data may now be outdated.[2] More recent studies including monitoring of postnatal weight gain and insulin-like growth factor 1 have had significant success in detecting ROP and offer a potentially safe way to identify a smaller 'at risk' population for screening.[3] Ethnicity also has an influence on the prevalence of ROP that may be related to average birth weight or due to the genetic polymorphisms seen in that ethnic group, for this reason it would be interesting to know the ethnicity of the infants presented in table 1.[4] Anecdotally, over the past 15 years in Bradford where the population is predominantly Asian, no babies have been treated for ROP who have weighed more than 1001 g.

Although the genetic susceptibility to ROP for the majority of cases still remains elusive, three of the four known genes implicated in familial exudative vitreoretinopathy (FEVR), NDP, FZD4 and LRP5 have polymorphisms that account for 10–12% of ROP.[5] For cases that fall outside the currently accepted 'at risk' guidelines (eg. case 10), it may be worth contemplating if in fact they have a clinically identical condition, FEVR, or at least a genetic basis for an increased susceptibility to develop abnormal retinal vasculature when exposed to environmental stressors (that may be tested for in future screening algorithms). For this reason, it may also be important to know the systemic clinical condition of the neonates presented during their inpatient stay.

Using our current guidelines, we are only treating approximately 10% of cases screened; even if this strategy allowed us to identify all cases, this would be enough reason to suggest a refinement of the current criteria. We feel that as our understanding of molecular genetics evolves, advances in neonatal care continues to improve together with changes in the UK population demographic re-examination of the screening criteria will become necessary.

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REFERENCES

1. **Durnian JM,** Clark DI. Retinopathy of prematurity: keeping the status quo. A case series detailing the importance of keeping the current guidelines for screening. *Br J Ophthalmol* 2010;**94**:1693–4.
2. **Fledelius HC.** Retinopathy of prematurity. Clinical findings in a Danish County 1982–87. *Acta Ophthalmol (Copenh)* 1990;**68**:209–13.
3. **Hard AL,** Lofqvist C, Fortes Filho JB, *et al*. Predicting proliferative retinopathy in a Brazilian population of preterm infants with the screening algorithm WINROP. *Arch Ophthalmol* 2010;**128**:1432–6.
4. **Ng YK,** Fielder AR, Shaw DE, *et al*. Epidemiology of retinopathy of prematurity. *Lancet* 1988;**2**:1235–8.
5. **Shastry BS.** Genetic susceptibility to advanced retinopathy of prematurity (ROP). *J Biomed Sci* 2010;**17**:69.

Simultaneous amniotic membrane transplantation in emergency penetrating keratoplasty: a therapeutic option for severe corneal ulcerations and melting disorders

In cases of severe corneal melting, immediate penetrating keratoplasty (PK) can be required but is accompanied by a high prevalence of complications due to ongoing inflammatory stimuli and wound-healing disorders. In these situations, the properties of amniotic membrane (AM) including promotion of epithelial healing as well as antiangiogenic, anti-infectious, antiscarring and immunemodulatory effects can be beneficial. In the context of PK and AM transplantation (AMT) different surgical

EDITOR'S CHOICE

Long-term results of autologous cultivated oral mucosal epithelial transplantation in the scar phase of severe ocular surface disorders

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ABSTRACT

Purpose To investigate the long-term outcome of autologous cultivated oral mucosal epithelial transplantation (COMET) for the treatment of the scar phase of severe ocular surface disorders.

Participants This study involved 19 eyes of 17 patients who received autologous COMET for total limbal stem-cell deficiency.

Methods Autologous cultivated oral mucosal epithelial sheets were created using amniotic membrane as a substrate. Clinical efficacy was evaluated by best-corrected visual acuity and visual acuity at the postoperative 36th month. The clinical results (clinical conjunctivalisation, corneal opacification, corneal neovascularisation and symblepharon formation) were evaluated and graded on a scale from 0 to 3 according to their severity. Clinical safety was evaluated by the presence of persistent epithelial defects, ocular hypertension and infections.

Results Autologous cultivated oral mucosal epithelial sheets were successfully generated for all 17 patients. All patients were followed up for more than 36 months; the mean follow-up period was 55 months and the longest follow-up period was 90 months. During the long-term follow-up period, postoperative conjunctivalisation and symblepharon were significantly inhibited. All eyes manifested various degrees of postoperative corneal neovascularisation, but it gradually abated and its activity was stable at 6 months after surgery. Best-corrected visual acuity was improved in 18 eyes (95%) during the follow-up periods, and visual acuity at the postoperative 36th month was improved in 10 eyes (53%).

Conclusions These long-term clinical results strongly support the conclusion that tissue-engineered cultivated oral mucosal epithelial sheets are useful in reconstructing the ocular surface of the scar phase of severe ocular surface disorders.

INTRODUCTION

Severe ocular surface disease (OSD), such as Stevens–Johnson syndrome (SJS) and ocular cicatricial pemphigoid (OCP), can be devastating and result in significant visual complications.[1–5] Since 1997, with the knowledge of tissue-specific stem cell behaviour and the development of tissue-engineering techniques, cultivated corneal limbal epithelial transplantation (CLET) has been shown to be a promising treatment modality for the management of severe OSD.[6–12] However, as most severe OSD is bilateral, surgeons were forced to use allograft donor cells, which subject the recipients to a high risk for allogeneic rejection and necessitate prolonged immunosuppression therapy. More recently, our experimental[13] and serial clinical studies[14–18] demonstrated the efficacy of autologous cultivated oral mucosal epithelial transplantation (COMET) for the treatment of severe OSD. Even though initial clinical results of COMET have been reported from several groups worldwide,[19 20] including our group, the long-term clinical assessments of COMET are entirely unknown and the feasibility of this technique still requires detailed investigation.

Here, we present the long-term clinical data on 19 eyes that received COMET, for which the mean follow-up period was 55 months; the longest follow-up period being 90 months. This study has important clinical implications and provides new information regarding the long-term visual results and survival of transplanted cultivated cells for the treatment of the scar phase of severe OSD cases.

MATERIALS AND METHODS

Subjects

All experimental procedures and clinical applications introduced in this study were approved by the Institutional Review Board for Human Studies of Kyoto Prefectural University of Medicine; prior informed consent was obtained from all patients in accordance with the tenets of the Declaration of Helsinki for research involving human subjects.

The study included 19 eyes from 17 patients with the scar phase of severe OSD who underwent ocular surface reconstruction with COMET at our hospital from August 2002 to January 2007, and who could be followed up for more than 36 months. In this study, to precisely examine the long-term clinical results of COMET for corneal surface reconstruction, we excluded the patients who received penetrating keratoplasty after the initial COMET and patients who received COMET for conjunctival fornix reconstruction. The patients included 7 males and 10 females; their ages ranged from 20 to 80 years (mean age: 54±21 years). The patients were followed up for a mean period of 55±17 months; the longest follow-up period was 90 months. All patients were diagnosed as totally stem-cell-deficient on the basis of complete disappearance of the palisades of Vogt and 360° of conjunctivalisation. The preoperative diagnosis was SJS in 11 eyes, OCP in 4 eyes, squamous cell carcinoma in 2 eyes, thermal or chemical injury in 1 eye and graft-versus-host disease in 1 eye. Tear production was diminished but not absent in all

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patients, as evidenced by the presence of a tear meniscus level with diminished tear-film break-up time.

Cultivation of oral mucosal epithelial sheets

We cultured human oral mucosal epithelial cells using a previously reported system.[14–18] Briefly, the presence of healthy oral mucosa was first confirmed by a dentist before biopsy. A small oral mucosal biopsy was performed under local anaesthesia. The oral epithelium was then incubated at 4°C for 5 h with 1.2 IU Dispase, followed by treatment with 0.05% Trypsin-EDTA solution for 10 min to separate the cells. The resultant oral epithelial cells ($1–2\times10^5$ cell/ml) were then seeded onto denuded amniotic membrane (AM) spread on the bottom of culture inserts and co-cultured with mitomycin C (MMC)-inactivated 3T3 fibroblasts. The culture medium consisted of defined keratinocyte growth medium (KGM: ArBlast Co., Ltd., Kobe, Japan) supplemented with 5% serum. The cultured cells were submerged in medium for 2 weeks and then air-lifted for 1–2 days by lowering the medium level.

Surgical procedure

The surgical procedure was as described in our previous report.[14–18] Briefly, we performed a 360° conjunctival peritomy 3 mm from the limbus and removed all perilimbal scarred or inflamed subconjunctival tissue down to bare sclera. The corneal pannus was completely removed by blunt dissection or superficial keratectomy using surgical scissors or a blade. The cultivated oral mucosal epithelial sheet was placed over the corneal surface and secured in place with 10-0 nylon sutures at the limbus. The integrity of the cultivated epithelium was confirmed by fluorescein staining at the end of the surgery, and the ocular surface was protected with a medical-use bandage contact lens (Plano B4). Postoperatively, three types of medical-use bandage contact lenses (Plano B4, Acuvue and O_2 Optics) were properly used within 1–3 months after surgery depending on the condition of the corneal surface.

Postoperatively, 0.3% ofloxacin and 0.1% dexamethasone eye drops were instilled four times a day. The doses were tapered to a maintenance dose of—two to three times a day after 2–3 months, depending on the severity of inflammation. Oral betamethasone (1 mg/day) and cyclosporine (100 mg/day) were administered to reduce inflammation and were tapered down and then stopped 1 month postoperatively.

Evaluation of clinical efficacy

Preoperative visual acuity (VA), postoperative best-corrected visual acuity (BCVA) and VA at the postoperative 36th month were measured, and the ocular surface was inspected with a slit-lamp microscope and fluorescence staining. The clinical results (clinical conjunctivalisation (eg, invasion of conjunctival tissue), corneal opacification, corneal neovascularisation and symblepharon formation) were evaluated by two ophthalmologists and graded on a scale from 0 to 3 according to their severity in accordance with our previously reported grading system.[21]

Evaluation of clinical safety

For the assessment of postoperative complications, the patient's eyes were carefully examined for persistent epithelial defects (PEDs), ocular hypertension and infections. Epithelial defects were considered persistent if they lasted for more than 4 weeks. Ocular hypertension was considered a postoperative complication if it had not been present preoperatively. When we clearly observed the clinical bacterial focus region in the cornea, we considered it a corneal infection.

RESULTS

There were no complications during or after the excision of the oral mucosa. Autologous cultivated oral mucosal epithelial sheets were successfully generated for all 17 patients, but one case was merely fair because only 70% of the entire cultivated epithelial sheet showed mature stratification as determined by fluorescein staining under a phase-contrast microscope and an operating microscope at the end of surgery. All eyes, including the eye transplanted with the sheet whose quality we judged as only fair by use of an inverted microscope, demonstrated total re-epithelialisation of the corneal surface within 2–7 days after surgery. Successful engraftment was initially achieved in all patients with none of the grafts sloughing off. Combined surgery was performed as follows: subconjunctival MMC treatment (17 eyes, 90%), amniotic membrane transplantation (AMT) (14 eyes, 74%) and cataract surgery (5 eyes, 26%).

Preoperative BCVA in our series was light perception, hand motion (HM) or finger counting (15 eyes, 79%) and worse than 20/500 (4 eyes, 21%). Postoperative VA (BCVA, and VA at the postoperative 36th month) is summarised in figure 1 and table 1. Postoperative visual recovery ranged from HM to 20/40; during the follow-up, BCVA was improved more than 2 lines in 15 eyes (79%) and VA at the postoperative 36th month was improved in 8 eyes (42%).

The clinical grading scores pertaining to conjunctivalisation, corneal opacity, corneal neovascularisation and symblepharon formation were evaluated, and it was found that during the long-term follow-up period, postoperative clinical conjunctivalisation

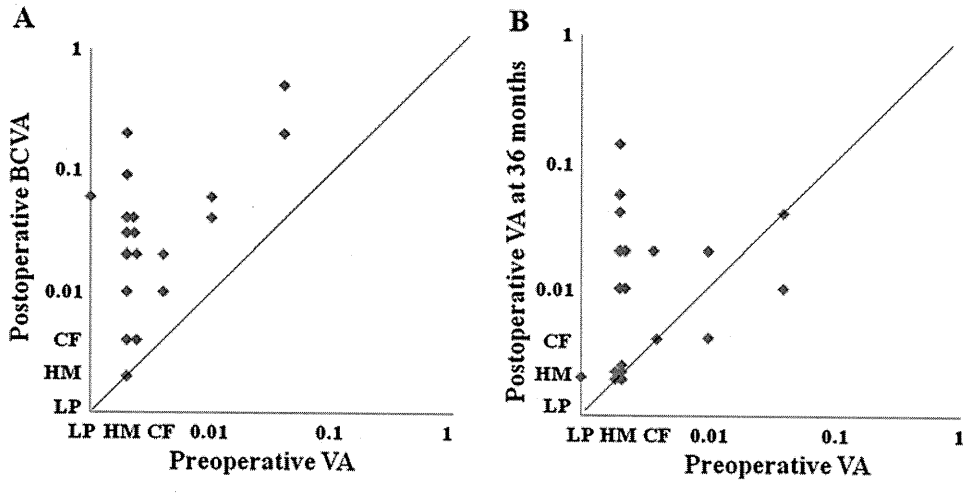

Figure 1 Postoperative best-corrected visual acuity (BCVA) (A) and visual acuity (VA) at the postoperative 36th month (B). The diagonal line indicates the values at which the preoperative and postoperative values for visual acuity were the same. CF: counting fingers; HM: hand motion; LP: light perception.

Table 1 Summary of postoperative VA

	Postoperative BCVA (%)	Postoperative VA at 36 months (%)
Improvement of VA (more than two lines)	79	42
Improvement of VA (more than one line)	95	53
No change	5	37
Decline	0	11

BCVA, best-corrected visual acuity; VA, visual acuity.

and symblepharon were significantly inhibited (figure 2). Corneal opacification tended to improve. All eyes manifested various degrees of superficial corneal vascularisation, but it gradually abated and its activity was comparatively stable from 6 months after surgery. We theorise that postoperative neovascularisation occurs because in vivo oral mucosa requires a vascular bed for maintenance.

Regarding the postoperative complications, a small but persistent epithelial defect was observed in 5–26% of the patients during the follow-up periods (table 2). Of the total 19 eyes, 7 eyes (37%) manifested PEDs once during the follow-up periods, while postoperative ocular hypertension was observed in 3 eyes (16%) (table 2). Although the intraocular pressure (IOP) of those three patients was occasionally high, they did not require glaucoma surgery. The occasional increase in IOP was mainly managed by the administration of carbonic anhydrase inhibitor (two eyes), or by the topical application of 0.05% latanoprost (one eye) or carteolol hydrochloride (one eye). Methicillin-resistant *Staphylococcus aureus* (MRSA) was the only cause of postoperative corneal infection (one eye), and that corneal infection was observed only within 6 months after transplantation (table 2).

The clinical progress of three representative patients with total limbal stem cell deficiency arising from SJS and idiopathic OSD is shown in figure 3. Before transplantation all eyes manifested severe destruction of the ocular surface with limbal stem cell deficiency (figure 3A,C,E). Postoperative appearance at 50 (figure 3B) and 71 (figure 3D) months shows a relatively smooth, epithelialised corneal surface with minimal corneal neovascularisation, scarring and inflammation. Postoperative appearance at 72 months (figure 3F) shows that due to the PED during the follow-up periods, postoperative corneal opacity was stronger than in other cases with modest neovascularisation, finally affecting the transparency of the cornea even though the ocular surface was relatively stable.

DISCUSSION

Ocular surface reconstruction for severe OSD continues to be one of the most challenging fields in ophthalmology. COMET is the most recent therapeutic method for the treatment of severe OSD, and this study provides new information regarding the long-term results of this new treatment. We found through our earlier preliminary and mid-term clinical results[14–18] that COMET is an efficacious treatment for severe OSD. The overall success rate, as measured by the improvement of BCVA and VA at the postoperative 36th month, was 95% and 53%, respectively. This success rate is similar to that of a previous report, and the patients who participated in this study were some of the most severe cases with their preoperative VA all being worse than 20/500, suggesting that our clinical results were fair and that COMET was useful for reconstructing the ocular surface of these patients with severe OSD.

In 1999, our group started the clinical application of allogeneic CLET and since 2002 we have been performing COMET for patients with severe OSD. Through these many years of clinical experience we have learned a great deal from our clinical and biological findings as follows: (1) Wearing a therapeutic soft contact lens in the early postoperative period is essential for the clinical success of COMET because it provides protection for the epithelial cells from mechanical ablation; we first used the relatively rigid-type soft contact lens (eg, the Plano B4), which is

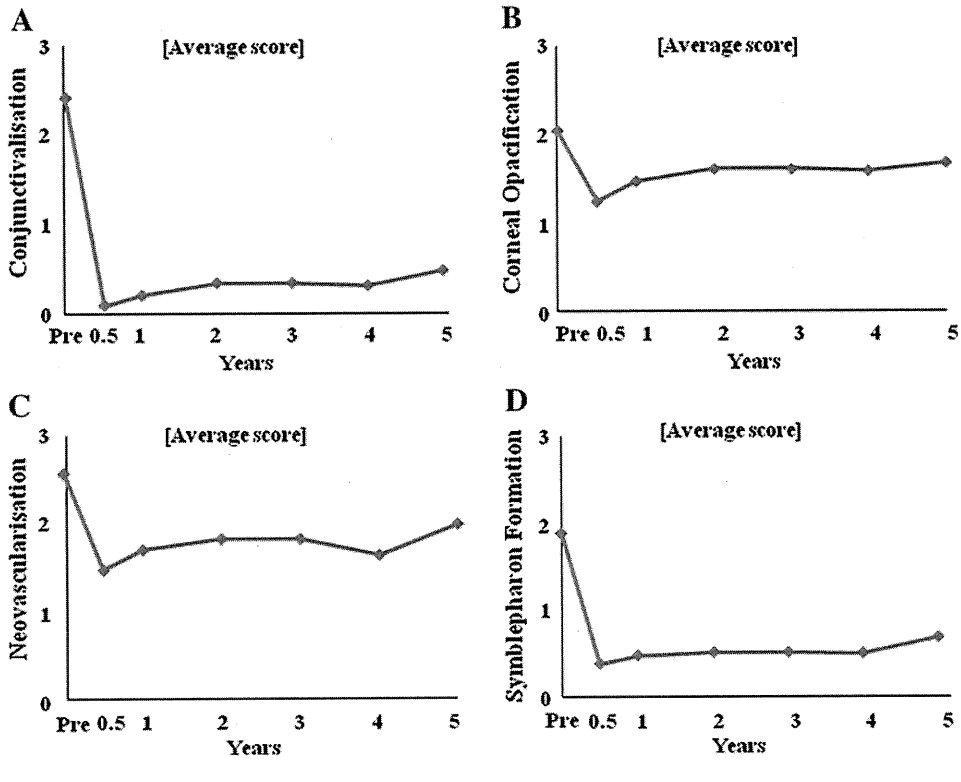

Figure 2 The results of the postoperative observation time course of clinical grading score regarding conjunctivalisation (A), corneal opacity (B), corneal neovascularisation (C), and symblepharon formation (D).

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Table 2 Time course of postoperative complications

	Pre	6M	12M	24M	36M	48M	60M
Persistent epithelial defect	5% (1/19)	26% (5/19)	5% (1/19)	5% (1/19)	5% (1/19)	8% (1/12)	0% (0/8)
Ocular hypertension	5% (1/19)	10% (2/19)	15% (3/19)	5% (1/19)	5% (1/19)	8% (1/12)	12% (1/8)
Infection	0% (0/19)	5% (1/19)	0% (0/19)	0% (0/19)	0% (0/19)	0% (0/12)	0% (0/8)

M, months.

difficult to take off, and then we used the highly hydrated-type soft contact lens (Acuvue, O_2 Optics) to improve the permeability of oxygen to the corneal surface and to prevent epithelial damage due to lack of oxygen. (2) Within 1 week of COMET, almost all patients encountered dry-eye conditions, the severity of which depended upon the individual patient. Therefore, artificial eye drops should be instilled frequently (from every 15 min to 2 h).

Even though the morphological appearance of a cultivated oral mucosal epithelial sheet is quite similar to that of a cultivated corneal limbal epithelial sheet, one of the most distinguishable characteristics of a cultivated oral mucosal epithelial sheet is its distinctive fluorescein staining pattern. From our clinical experience, its staining pattern is more like that of superficial punctate keratopathy than conjunctival epithelium, but a strict discrimination between the two is somewhat difficult to observe by slit-lamp examination. Although the transplanted cultivated oral mucosal epithelial sheets in this study retained their transparency, there was a slightly high amount of light-scattering under the slit-lamp microscope examination, with or without fluorescein staining, thus affecting the postoperative corneal opacity and resulting in a BCVA of 20/40 in the 19 eyes treated by COMET. For most eyes, the BCVA was between 20/2000 and 20/200, suggesting that light-scattering on the transplanted oral mucosal epithelium on the cornea reached the level that controls VA at around 20/500. In contrast, the BCVA of some CLET patients reached 20/20, indicating that the biological character of the cells clearly affects the quality of VA.

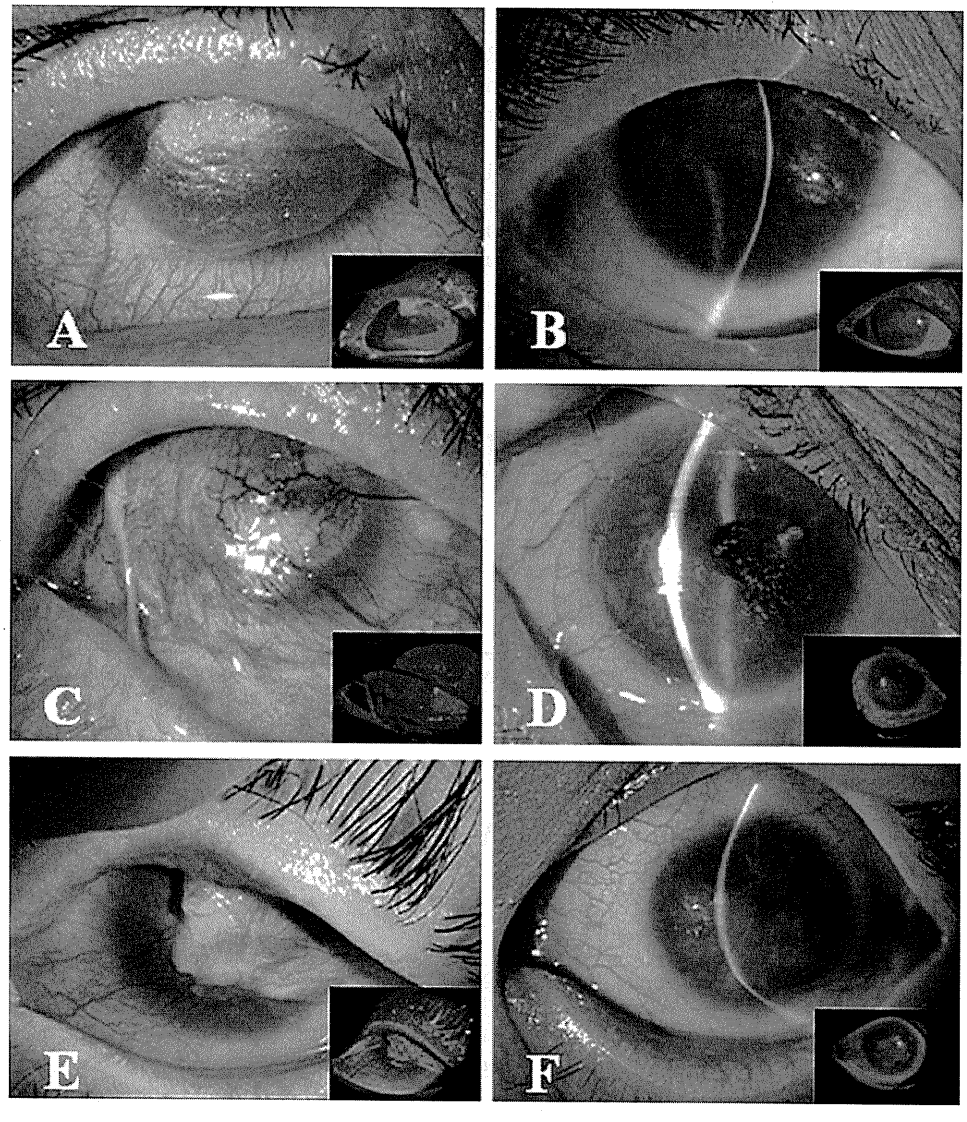

Figure 3 The clinical progress of three representative patients with severe OSD arising from SJS (A, B), idiopathic OSD (C, D) and SJS (E, F). Before transplantation all eyes manifested severe destruction of the ocular surface with limbal stem cell deficiency (A, C and E). The postoperative appearance at 50 (B) and 71 (D) months shows a relatively smooth, epithelialised corneal surface with minimal corneal neovascularisation, scarring and inflammation. The postoperative appearance at 72 months (F) shows that due to the persistent epithelial defect during the follow-up periods, postoperative corneal opacity is somehow remarkable with modest neovascularisation, finally affecting the transparency of the cornea even though the ocular surface is relatively stable. OSD, ocular surface disease; SJS, Stevens–Johnson syndrome.

The patients with OSD reported here were the most severe cases we encountered, necessitating the reconstruction of not only the corneal surface but also the surrounding ocular surface. Of the 19 eyes in this study, 90% and 74% of the cases received treatment by MMC and AMT, respectively, to prevent the postoperative proliferation activity of the subconjunctival tissue and to reconstruct the ocular surface including the conjunctival fornix. After COMET, transplanted cultivated oral epithelial cells were always observed to migrate outwards on the AM, ultimately covering the complete ocular surface. Through the simultaneous combination of these procedures, postoperative symblepharon formation was significantly inhibited during the long-term follow-up and we believe that this is the one of the most beneficial advantages of COMET. In addition, of the 19 eyes, 26% of the cases simultaneously received cataract surgery to improve the VA. Even though the intraocular visibilities in patients with OSD were often very bad, we developed the surgical technique as a step-by-step process and can now perform it safely through the use of a surgical slit-lamp microscope and indocyanine green staining of the anterior lens capsule.

We carefully assessed the clinical safety of COMET and found that postoperative PED sometimes occurred in our series. Of the 19 eyes, PED occurred in 7 eyes (37%) at least once during the long-term follow-up. Of those seven eyes, five eyes (71%; all with SJS) were systemic primary OSD. It has been reported that the health of the oral mucosal epithelium in vivo depends on the existing diseases.[22] Even though we were able to generate a cultivated oral mucosal epithelial sheet from systemic primary OSD, whose morphological features are quite similar to in vivo corneal epithelium, the biological ability of the oral epithelium cells may potentially be damaged in these patients. This issue is currently under investigation in our laboratory.

Of the 19 eyes, ocular hypertension was observed in a total of 3 eyes (16%) during the postoperative follow-up period. Since the transplanted cultivated sheets were not completely identical to in vivo epithelium, care must be taken in regard to postoperative epithelial damage caused by the use of antiseptic eye drops. Thus, a major clinical point is that in two eyes in this study, the occasional increase in IOP was found to be better managed by the administration of a carbonic anhydrase inhibitor.

In our series, postoperative corneal infections were relatively few (one eye, 5%) as compared to allogeneic CLET or limbal transplantation,[9 23] simply because COMET is an autologous transplantation and patients did not need the intensive, prolonged postoperative immunosuppressant therapy, thus resulting in the avoidance of an immunocompromised state. Interestingly, in view of the findings in this study and our recent clinical experiences, the postoperative corneal infection in our cases mainly occurred within 6 months after surgery, and all of those cases were systemic OSD patients. Furthermore, all of those cases were detected to be caused by MRSA.[24] It has been reported that MRSA is frequently detected from the ocular surface in patients with SJS as compared with normal subjects; therefore, it is important to carefully observe the corneal surface in the early postoperative periods, especially in patients with SJS.

In conclusion, this is the first study that demonstrates the long-term clinical results of COMET for ocular surface reconstruction in the treatment of the scar phase of severe OSD. We found that COMET permits sustained reconstruction of the ocular surface epithelium in many eyes with severe OSD. The management of postoperative PED and neovascularisation may further increase the efficacy of this type of transplantation.

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Competing interests None.

Ethics approval This study was conducted with the approval of the Institutional Review Board for Human Studies of Kyoto Prefectural University of Medicine.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. **Shapiro MS,** Friend J, Thoft RA. Corneal re-epithelialization from the conjunctiva. *Invest Ophthalmol Vis Sci* 1981;**21**:135—42.
2. **Dua H,** Forrester JV. The corneoscleral limbus in human corneal epithelial wound healing. *Am J Ophthalmol* 1990;**110**:646—56.
3. **Tsai RJF,** Sun TT, Tseng SC. Comparison of limbal and conjunctival autograft transplantation in corneal surface reconstruction in rabbits. *Ophthalmology* 1990;**97**:446—55.
4. **Thoft RA.** Keratoepithelioplasty. *Am J Ophthalmol* 1984;**97**:1—6.
5. **Kenyon KR,** Tseng SC. Limbal autograft transplantation for ocular surface disorders. *Ophthalmology* 1989;**96**:709—23.
6. **Pellegrini G,** Traverso CE, Franzi AT, *et al*. Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. *Lancet* 1997;**349**:990—3.
7. **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.
8. **Schwab IR,** Reyes M, Isseroff RR. Successful transplantation of bioengineered tissue replacements in patients with ocular surface disease. *Cornea* 2000;**19**: 421—6.
9. **Koizumi N,** Inatomi T, Suzuki T, *et al*. Cultivated corneal epithelial stem cell transplantation in ocular surface disorders. *Ophthalmology* 2001;**108**:1569—74.
10. **Rama P,** Bonini S, Lambiase A, *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.
11. **Grueterich M,** Espana EM, Touhami A, *et al*. 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**:1547—52.
12. **Shimazaki J,** Aiba M, Goto E, *et al*. Transplantation of human limbal epithelium cultivated on amniotic membrane for the treatment of severe ocular surface disorders. *Ophthalmology* 2002;**109**:1285—90.
13. **Nakamura T,** Endo K, Cooper LJ, *et al*. The successful culture and autologous transplantation of rabbit oral mucosal epithelial cells on amniotic membrane. *Invest Ophthalmol Vis Sci* 2003;**44**:106—16.
14. **Nakamura T,** Inatomi T, Sotozono C, *et al*. Transplantation of cultivated autologous oral mucosal epithelial cells in patients with severe ocular surface disorders. *Br J Ophthalmol* 2004;**88**:1280—4.
15. **Inatomi T,** Nakamura T, Koizumi N, *et al*. The mid-term results of ocular surface reconstruction using cultivated autologous oral mucosal epithelial transplantation. *Am J Ophthalmol* 2006;**141**:267—75.
16. **Inatomi T,** Nakamura T, Kojyo M, *et al*. Ocular surface reconstruction with combination of cultivated autologous oral mucosal epithelial transplantation and penetrating keratoplasty. *Am J Ophthalmol* 2006;**142**:757—64.
17. **Ang LPK,** Nakamura T, Inatomi T, *et al*. Autologous serum-derived cultivated oral epithelial transplants for severe ocular surface disease. *Arch Ophthalmol* 2006;**124**:1543—51.
18. **Nakamura T,** Inatomi T, Cooper LJ, *et al*. Phenotypic investigation of human eyes with transplanted autologous cultivated oral mucosal epithelial sheets for severe ocular surface diseases. *Ophthalmology* 2007;**114**:1080—8.
19. **Nishida K,** Yamamoto M, Hayashida Y, *et al*. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. *N Engl J Med* 2004;**351**:1187—96.
20. **Satake Y,** Dogru M, Yamane GY, *et al*. Barrier function and cytologic features of the ocular surface epithelium after autologous cultivated oral mucosal epithelial transplantation. *Arch Ophthalmol* 2008;**126**:23—8.
21. **Sotozono C,** Ang LPK, Koizumi N, *et al*. New grading system for the evaluation of chronic ocular manifestations in patients with Stevens-Johnson syndrome. *Ophthalmology* 2007;**114**:1294—302.
22. **Scully C,** Bagan J. Oral mucosal diseases: erythema multiforme. *Br J Oral Maxillofac Surg* 2008;**46**:90—5.
23. **Tsubota K,** Satake Y, Kaido M, *et al*. Treatment of severe ocular-surface disorders with corneal epithelial stem-cell transplantation. *N Engl J Med* 1999;**340**: 1697—703.
24. **Sotozono C,** Inagaki K, Fujita A, *et al*. Methicillin-resistant Staphylococcus aureus and methicillin-resistant Staphylococcus epidermidis infections in the cornea. *Cornea* 2002;**21**(7 Suppl):S94—101.