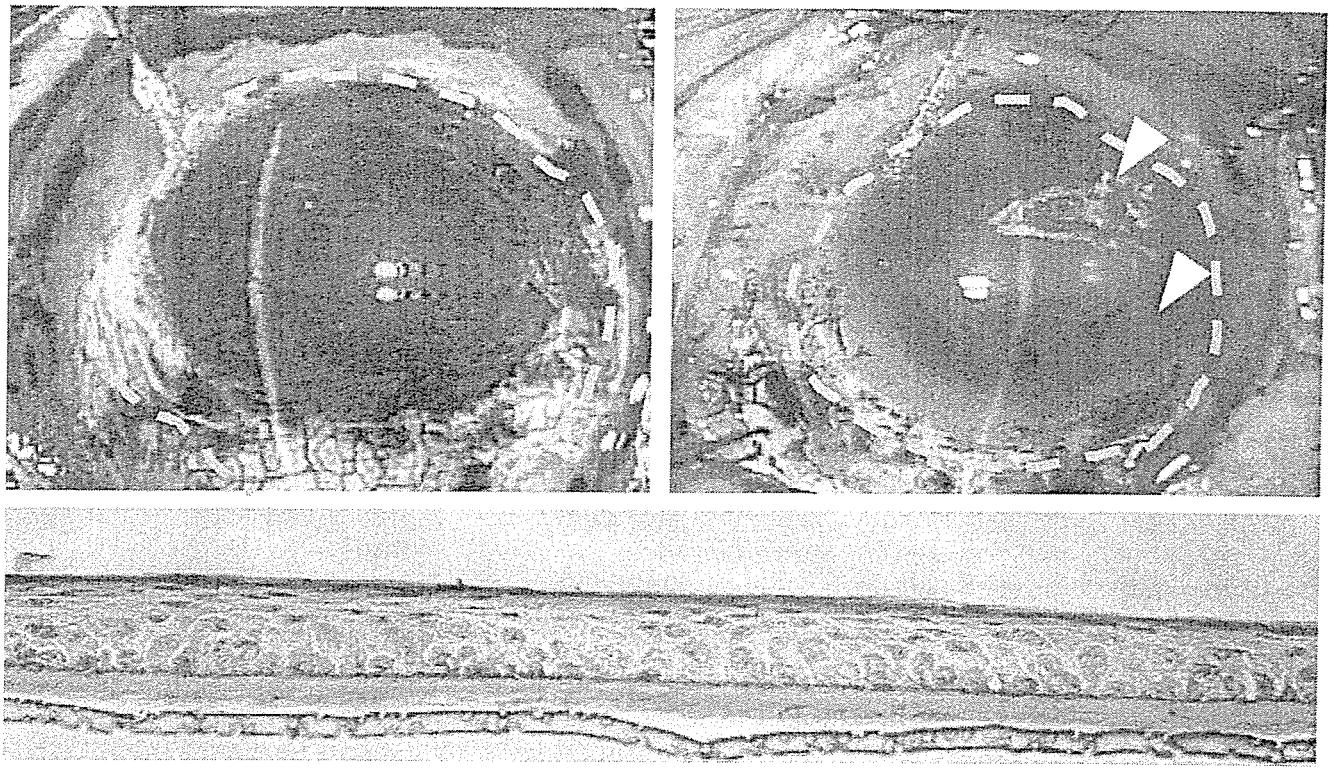

FIGURE 1. The integrity of all transplanted cultivated oral mucosal epithelial sheets confirmed by fluorescein staining at the end of ocular surface reconstruction. The yellow broken line encompasses negatively-stained cultivated stratified oral mucosal epithelium. Arrows indicate the region harboring the epithelial defect in the sheet that was considered to be of only fair quality before grafting. Of the 15 sheets, 14 (93.3%) were of excellent quality and without epithelial defects (Top left); one case was scored as fair with some epithelial defects (Top right). Histologic examination using hematoxylin and eosin staining revealed good stratification throughout the entire sheet (Bottom). EP: cultivated oral mucosal epithelium; AM: amniotic membrane; CI: culture insert.

using a 2-hour incubation at 37°C with ethylene diamine tetraacetic acid (EDTA) 0.02% to loosen cell adhesion. This was followed by gentle scraping with a cell scraper.

The presence of healthy oral mucosa in our patients was confirmed by a dentist before biopsy. All patients were monitored to confirm their adherence to required tooth-decay treatment, their abstinence from alcohol or tobacco use, and their regular performance of tooth brushing and iodine gargling. Under local anesthesia, oral mucosal biopsy specimens, each measuring approximately 2 to 3 mm^2, were obtained 2 to 3 weeks before the planned transplantation procedure. Submucosal connective tissues were removed with scissors to the extent possible, with the resulting samples being cut into small explants that were then immersed three times (10 minutes, room temperature) in phosphate-buffered saline solution containing antibiotics (50 IU/ml penicillin-streptomycin and 5 μg/ml amphotericin B). The explants were then incubated at 37°C for 1 hour with 1.2 IU dispase as previously described[13] and treated with trypsin-EDTA 0.05% solution for 10 minutes at room temperature to separate the cells. Enzyme activity was stopped by washing with culture medium comprised of DMEM and Ham's F12 medium (1:1) containing insulin (5 μg/ml), cholera toxin (0.1 nmol/l), human recombinant epidermal growth factor (10 ng/ml), and penicillin-streptomycin (50 IU/ml). In cultures for eyes no. 1 to 9, the medium also contained 10% fetal calf serum. In cultures for eyes no. 10 to 15, we included 10% autologous serum. The oral mucosal epithelium was then seeded onto denuded AM spread on the bottom of culture inserts, and cocultured with mitomycin-C (MMC)-inactivated 3T3 fibroblasts. The culture was submerged in medium for 2 weeks and then exposed to air by lowering the level of the medium (air lifting) for 1 to 2 days. Cultures were incubated at 37°C in a 5% CO_2-95% air incubator; the medium being changed daily. Baseline data on the oral mucosal epithelial cultures are summarized in Table 1.

• SURGICAL PROCEDURE FOR OCULAR SURFACE RECONSTRUCTION USING CULTIVATED AUTOLOGOUS ORAL MUCOSAL EPITHELIAL TRANSPLANTATION: The surgical procedure was as described in our previous report.[4] Stated briefly, after a 360-degree conjunctival peritomy, we either scraped the area with the epithelial defect, or completely removed the conjunctivalized tissue by thin superficial keratectomy on the corneal surface. Subconjunctival spaces were treated with MMC 0.04% for 5 minutes, followed by a vigorous washing with saline. Then,

TABLE 2. Characteristics of Cases and Clinical Outcome of Patients With Oral Mucosal Epithelial Culture Reconstruction

Case	Age/Gender	Eye	Disease	Prior Op	Combined Op	Visual Acuity			Complication	Follow-up (mos)
						Pre Op	Post Op	Last VA		
1	33/M	OS	Chemical (acute)	AMT		HM	20/200	20/40		34
2	33/M	OD	Chemical (acute)	AMT		HM	HM	HM		34
3	27/M	OS	Chemical (chronic)	None	AMT	HM	CF	HM		32
4	24/M	OS	SJS	CCET		HM	20/2000	CF	ED	29
5	14/F	OS	SJS	None		CF	20/1000	20/1000		28
6	24/M	OD	SJS	CCET		HM	20/2000	CF	ED	28
7	65/F	OD	SJS	AMT + KEP	PEA + IOL	CF	20/400	20/500	ED	26
8	61/F	OD	OSD	AMT + LT	PEA + IOL	HM	20/500	20/800		23
9	69/M	OD	Chemical (chronic)	PK	PK*	HM	HM	20/50		18
10	65/F	OS	SJS	None	PEA + IOL	HM	20/320	20/320		12
11	70/M	OS	SJS	None	PK*	HM	HM	20/1000		11
12	67/F	OD	SJS	None	PEA + IOL	HM	20/2000	20/2000	ED	8
13	29/M	OD	Thermal (acute)	None	Lid	20/500	20/1000	20/32	ED	8
14	81/F	OS	pOCP	None	PEA + IOL + PPV	20/400	20/63	20/63		6
15	64/M	OD	Chemical (acute)	None	PEA + IOL + Lid	20/500	20/250	20/500		3

AMT = amniotic membrane transplantation; CCET = cultivated corneal epithelial transplantation; CF = count finger; Chemical = chemical injury; ED = epithelial defect; HM = hand motion; IOL = intraocular lens; KEP = keratoepithelioplasty; Lid = lid plastic surgery; LT = limbal transplantation; OSD = idiopathic ocular surface disorder; PEA = phacoemulsification; PK = penetrating keratoplasty; pOCP = pseudo-ocular cicatricial pemphigoid; PPV = pars plana vitrectomy; SJS = Stevens-Johnson syndrome; Thermal = thermal injury.

*Two cases received PK after primary surgery.

the cultivated autologous oral mucosal epithelial sheet in a culture dish was cut with a 19-mm diameter trephine, transferred onto the corneal surface, and sutured with 10-0 nylon. The integrity of the cultivated epithelium was confirmed by fluorescein staining at the end of surgery (Figure 1), and the ocular surface was protected with a medical-use contact lens.

• CLINICAL EVALUATION: Preoperative and postoperative best-corrected visual acuity was measured, and ocular surface manifestations were inspected with a slit-lamp microscope and fluorescein staining. Corneal superficial vascularization was monitored photographically and graded according to extent and intensity, where grade 1 indicates peripheral vascularization, grade 2 peripheral and midperipheral vascularization, grade 3 modest vascularization involving the entire cornea, and grade 4 massive vascularization of the entire cornea.

RESULTS

• CULTIVATED AUTOLOGOUS ORAL MUCOSAL EPITHELIAL SHEETS: There were no complications during or after the excision of oral mucosa. Cell suspensions of approximately 1×10^5 seeded oral mucosal epithelial cells began to form colonies on the denuded AM within 3 days. After 5 to 8 days in culture, a confluent primary culture of oral mucosal epithelial cells was established on the whole AM. After 2 weeks, the cultivated oral mucosal epithelium consisted of five to six cell layers and was similar to the cultivated corneal epithelial sheets we reported previously.[4,13] The oral mucosal epithelial sheet was composed of a well-conserved basal layer formed by cuboidal cells, several suprabasal cell layers, and flat apical cell layers (Figure 1). In 14 of 15 instances, the quality of the cultivated epithelial sheets was excellent. In one instance (Case 6), it 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 (Table 1, Figure 1).

• CLINICAL OUTCOMES: All eyes, including the eye transplanted with the sheet whose quality we judged as only fair, demonstrated total re-epithelialization of the corneal surface 2 days after surgery. During the follow-up period, in 10 of 15 eyes the ocular surface grafted with cultivated autologous oral mucosal epithelial sheets remained silent and fairly transparent. However, five eyes, including four with severe SJS, developed small but long-standing epithelial defects; two eyes proceeded to be completely healed by adjacent oral mucosal epithelium, one eye demonstrated conjunctival replacement, and the other two eyes required reoperation. Except for the latter two eyes, all ocular surfaces became stable without any

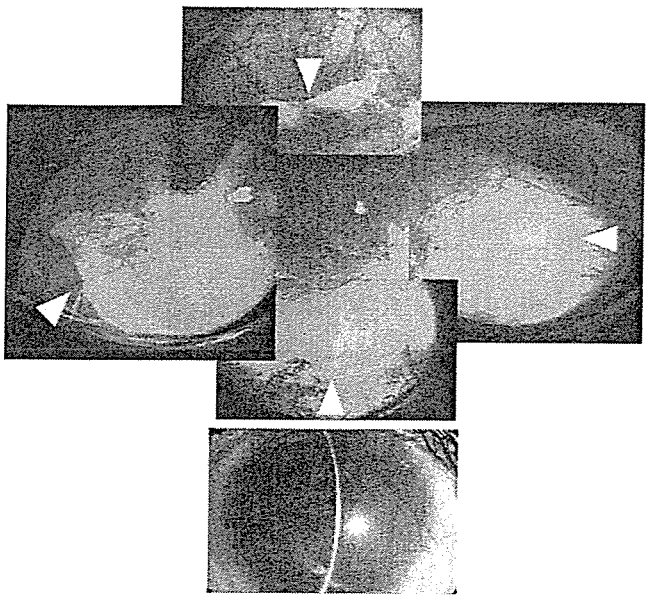

FIGURE 2. The clinical appearance of case 1 at 34 months after cultivated autologous oral mucosal epithelial transplantation. Fluorescein staining confirms the long-term survival of oral mucosal epithelium identified by the different levels of staining density. Arrows indicate the margin of the outgrowth of survived oral mucosal epithelium (Top). Slit-lamp photograph showing the appropriately resurfaced cornea. Note the modest vascularization involving the entire cornea beneath the amniotic membrane sheet and the preexisting corneal stromal opacity (Bottom).

major postoperative complications such as microbial infection or secondary glaucoma (Table 2).

Cultivated corneal epithelial stem cell sheets and ectopically surviving cultivated oral mucosal epithelial sheets are somewhat different in terms of their fluorescein staining patterns at the apical cell surface. In fact, regenerating epithelium that had originated from cultivated oral mucosal epithelium was clearly demarcated from adjacent conjunctival epithelium even as late as 34 months after surgery, the longest follow-up period in this series. This observation strongly suggests the long survival and epithelial supply of presumed oral mucosal epithelial stem cells (Figure 2).

Preoperative best-corrected visual acuity in our series was hand motion (HM) or counting fingers (12 eyes), 20/500 (two eyes), and 20/400 (one eye). Postoperative visual recovery ranged from HM to 20/32; best-corrected visual acuity was improved by more than 2 lines in 10 eyes (67%) at 3 months, and in 10 eyes (67%) at their latest follow-up examination. Three eyes with severe corneal opacity were scheduled for ocular surface reconstruction before penetrating keratoplasty. In cases 9 and 11, we performed a triple procedure with penetrating keratoplasty at 5 and 6 months after the ocular surface reconstruction procedure, respectively; visual acuity achieved in these two eyes was 20/50 and 20/1000. Of the 15 eyes, six were treated with cataract surgery immediately after the removal of ocular surface scarring using either a surgical slit-lamp or a special lighting device, and two eyes were treated with eyelid plastic surgery for entropion attributable to the primary injury (Table 2).

CASE REPORTS

FIGURES 3 AND 4, SHOW REPRESENTATIVE CASES OF CULTIvated autologous oral mucosal epithelial transplantation.

• CASE 1: A 33-year-old man in the acute phase of alkali injury graded IIIb with severe corneal stromal opacity in March 2002. AM transplantation was initially performed to cover the total damaged corneal surface, however, persistent corneal epithelial defect and severe inflammation prolonged for more than 1 month. Cultivated autologous oral mucosal epithelial transplantation was performed on June 24, 2002. Postoperatively, the ocular surface showed stabilized epithelialization with peripheral corneal vascularization (Figure 3). Even after 34 months of follow-up, surviving oral mucosal epithelium was distinguishable from conjunctival epithelium. The latest visual acuity was maintained at 10/20.

• CASE 5: A 14-year-old girl in the chronic phase of SJS with severe symblepharon over the cornea. The primary SJS occurred at the age of 5. The ocular surface was totally conjunctivalized with severe symblepharon without any surgeries. The ocular surface was reconstructed using cultivated oral mucosal epithelial transplantation, and the postoperative corneal surface was maintained fairly transparent. Best-corrected visual acuity improved from counting fingers to 20/1000 although the damaged corneal stroma was somewhat opaque (Figure 4).

• CASE 8: A 61-year-old woman with limbal deficiency of unknown etiology following AM transplantation and conventional allogeneic limbal transplantation. Primary surgery was performed in November 2000, but subsequent failure resulted in total conjunctivalization. After removal of scarred tissue and previously transplanted lenticles, the ocular surface was covered with a cultivated oral mucosal epithelial sheet. Postoperatively, the corneal surface showed complete epithelialization with minimal vascularization; some calcium deposits were observed (Figure 4).

• CASE 10: A 65-year-old woman with SJS. The primary SJS occurred at the age of 28. Visual acuity was reduced to CF, because of the conjunctivalization and the progression of cataract. Ocular surface was reconstructed in April 2004 using cultivated oral mucosal epithelial transplantation and cataract surgery. Postoperatively, the ocular surface was stable and transparent (Figure 4). Visual acuity improved to 20/320.

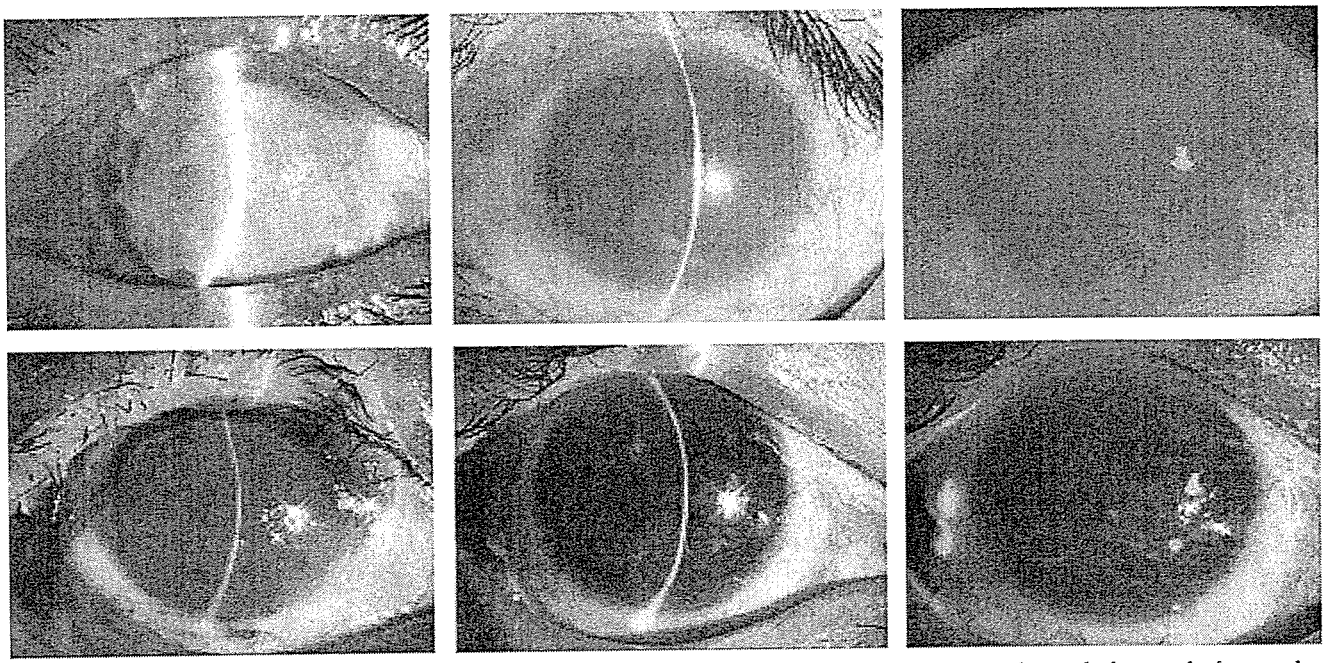

FIGURE 3. Slit-lamp photographs of two patients in the acute phase of chemical/thermal injury shown before and after ocular surface reconstruction using cultivated oral mucosal epithelial transplantation. Case 1 (33-year-old man): acute phase of alkali injury graded IIIb with severe corneal stromal opacity. (Top left) The ocular surface in preoperative condition. (Top center) Postoperative condition. (Top right) After fluorescein staining. Case 13 (29-year-old man): acute phase of thermal injury with total corneal stem-cell loss and a persistent epithelial defect. (Bottom left) The ocular surface in preoperative condition. (Bottom center) Postoperative condition. (Bottom right) After fluorescein staining.

• CASE 13: A 29-year-old man in the acute phase of thermal injury with total corneal stem cells loss and a persistent epithelial defect. He was injured in July 2004, and a persistent epithelial defect prolonged for more than 1 month. Simultaneously, progression of cicatrisation was observed. Therefore, we performed cultivated oral mucosal epithelial transplantation, and the ocular surface became stable after combined eyelid plastic surgery for cicatricial entropion (Figure 3).

• NEOVASCULARIZATION: All eyes grafted with cultivated oral mucosal epithelial sheets manifested various degrees of superficial corneal vascularization between the AM and corneal stroma. Preoperatively, most of the corneas had been covered with highly vascularized conjunctiva and had been given a grade of 4. Sparse or modest peripheral vascularization began after the first postoperative month (grade 1 to 2); in most cases, vascularization gradually progressed toward the center and peaked at 3 to 6 months. Although all grafted eyes manifested some degree of neovascularization, it gradually abated and over time it ceased to interfere markedly with visual function. At the 1-year follow-up, the neovascular formations were stable and none of the grafted eyes converted to their preoperative condition or exhibited oral mucosal tissue characteristics (Figure 5).

DISCUSSION

THIS MIDTERM STUDY DEMONSTRATES THE EFFECTIVENESS of cultivated autologous oral mucosal epithelial sheet transplantation and supports our earlier, preliminary report[14] by documenting multiple successful clinical results. According to their preliminary clinical study, Nishida and associates,[15] who grafted oral mucosal epithelial cell sheets cultured by methods different from ours,[13,14] also obtained positive results. This suggests that the transplantation of cultivated autologous oral mucosal epithelial sheets holds promise as a novel surgical treatment for severe ocular surface disorders such as SJS, ocular cicatricial pemphigoid, and chemical injury.

In the course of postoperative follow-up, their distinctive fluorescein staining pattern makes it easy to distinguish transplanted cultivated oral epithelial cell sheets from surrounding conjunctival epithelium. The staining pattern of epithelial cells of cultivated oral mucosal epithelial cell origin is more like that of superficial punctate keratopathy than conjunctival epithelium. Judging from their fluorescein staining at 2 days after surgery, with the exception of the sheet whose quality was considered only fair at the time of transplantation, almost all of the transplanted epithelial cells had attached on the cornea. In fact, histologically, the thriving oral mucosal epithelium at

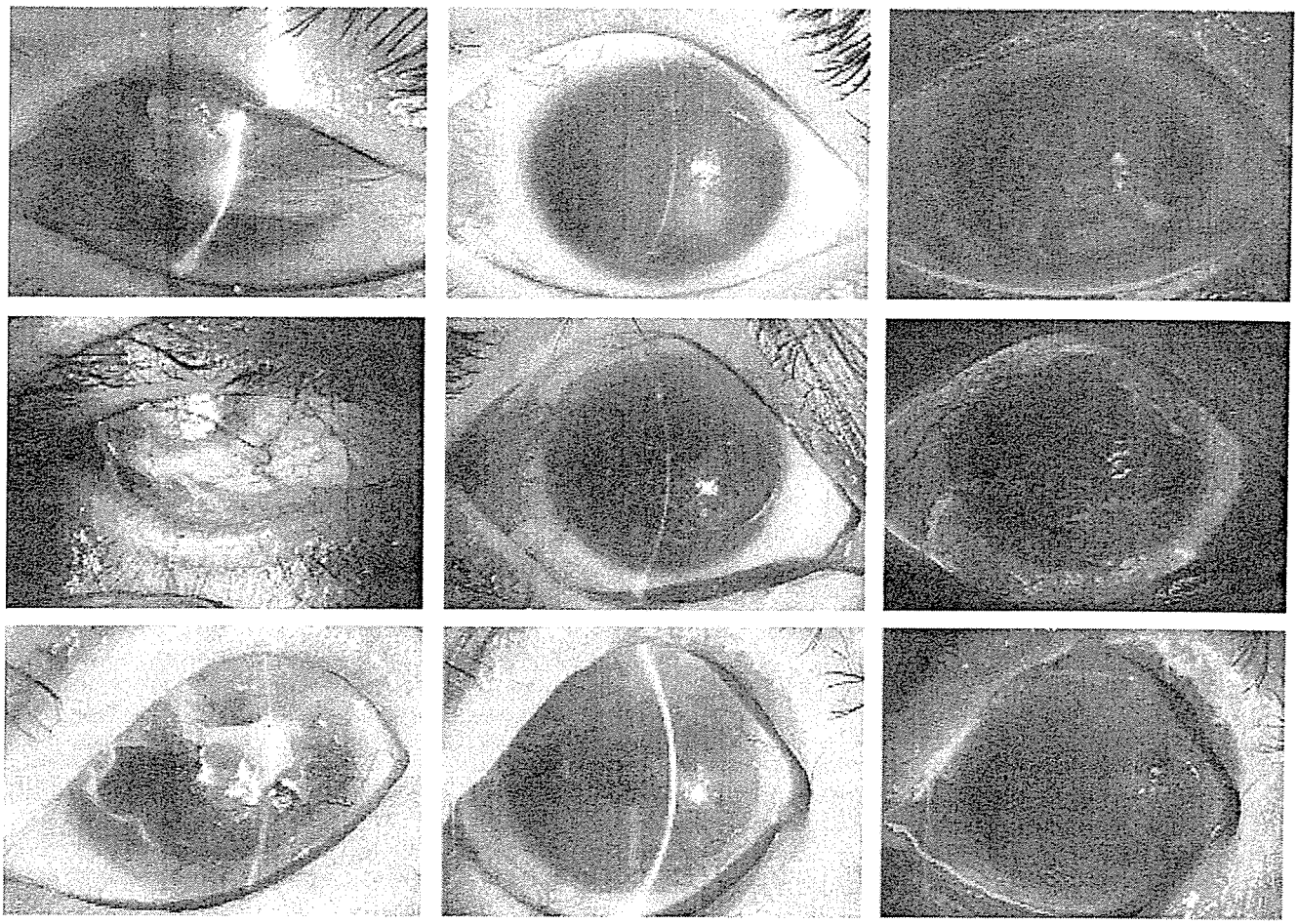

FIGURE 4. Slit-lamp photographs of three patients in the chronic phase of ocular surface disorders shown before and after ocular surface reconstruction using cultivated oral mucosal epithelial transplantation. Case 5 (14-year-old girl): chronic phase of SJS with severe symblepharon over the cornea. (Top row left) Preoperative condition. (Top row center) Postoperative condition. (Top row right) After fluorescein staining. Case 8 (61-year-old woman): limbal deficiency of unknown etiology. (Middle row left) Preoperative condition. (Middle row center) Postoperative corneal surface. (Middle row right) After fluorescein staining. Case 10 (65-year-old woman): chronic phase of SJS. (Bottom row left) Preoperative condition. (Bottom row center) Postoperative corneal surface. (Bottom row right) After fluorescein staining.

the central cornea that was removed at the time of penetrating keratoplasty (6 months after transplantation) was nonkeratinized stratified epithelium similar to corneal epithelium (data not shown). In the case followed for the longest period (34 months, Case 1), fluorescein staining results suggest that the cultivated oral mucosal epithelium cell sheet covered not only the entire cornea but also an area up to a few mm beyond the cornea. Although the transplanted epithelial sheets retained their transparency, there was a slight hazing, and the maximum corrected visual acuity we were able to obtain in our 15 eyes was 20/32. For most eyes, it was between 20/2000 and 20/32, suggesting the potential of visual recovery through the survived oral mucosal epithelium on the cornea may be around 20/200. This issue is currently under investigation at our laboratory.

The health of the oral mucosal epithelium in vivo depends on the existing disease. Patients with SJS always manifest mucosal epithelial damage in the acute phase. Ocular cicatricial pemphigoid, a type of mucous membranous pemphigoid, may also affect the oral mucosa. However, we were able to generate transplantable sheets from all 12 patients. In four instances, the transplantation of cultivated epithelium from patients with SJS resulted in small persistent epithelial defects, possibly because the oral mucosal epithelium was damaged. Alternatively, chronic ocular surface abnormalities may be different from other primary disorders. Although there is currently no solid evidence for the presence of stem cells in the human oral cavity, we posit that these cells are distributed as diffusely in the oral mucosal epithelium as in the human epidermis and conjunctival epithelium, and that oral mucosal epi-

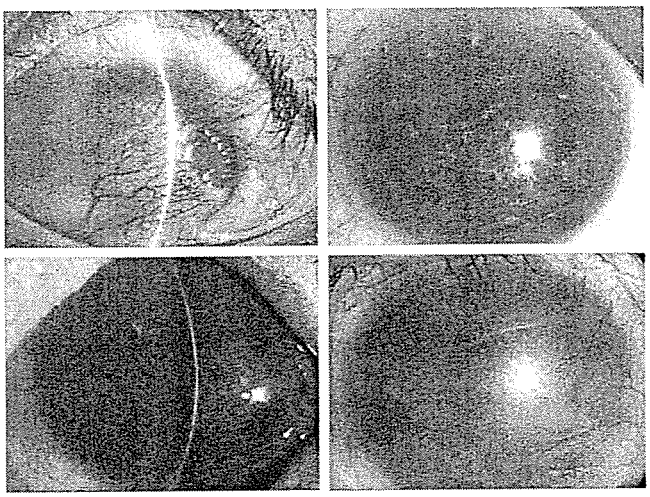

FIGURE 5. Slit-lamp photographs showing vascular formations after the transplantation of cultivated autologous oral mucosal epithelial sheets. (Top left) Preoperatively, most of the cornea manifested highly-vascularized conjunctivalization (case 3, grade 4). At the 1-year follow-up, vascular formations had abated and stabilized at grades 1 to 3. (Top right) Case 8 showed grade 1, (Bottom left) case 10 showed grade 2, and (Bottom right) case 1 showed grade 3.

thelial stem cells were present and impaired in these cases. Recently, Hayashida and associates[16] demonstrated p63 and β1-integrin positivity within the oral mucosa of rabbits, implying the presence of stem cells of oral mucosal epithelium in the oral cavity. In humans, we have a speculation that stem cells of oral mucosal epithelium may be diffusely located, similar to the rabbit study. This issue is also being investigated to rule out other factors in our laboratory.

In contrast to cultivated corneal epithelial stem cell transplants, the grafting of tissue-engineered oral mucosal epithelial cell sheets resulted in neovascularization in the superficial cornea. This suggests the presence of angiogenic activity whose level varies depending on the disorder and renders neovascularization inevitable. Transplanted buccal mucosa including subepithelial tissue survives by vessel recanalization. Gipson and associates,[18] who transplanted rabbit oral mucosal epithelium to the ocular surface, peeled the oral mucosal epithelial sheets by using dispase; their exfoliate transplantation results revealed vascularization. Tissue-engineered oral mucosal epithelial sheets may have weak, vascularization-inducing angiogenic activity. In fact, we found that some angiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF) are present. Conversely, our preliminary data demonstrated that one antiangiogenic factor, thrombospondin 1, appeared to be expressed in a low level in cultivated oral mucosal epithelial cells, which may be a possible explanation for the induction of neovascularization. (data not shown) We are investigating the basis of our highly interesting observation that different eyes manifested different degrees of vascularization that tended to peak at 3 to 6 months post-transplantation and declined thereafter. Thus, from the point of long-term ocular surface rehabilitation in severe cases, modest corneal neovascularization can be expected not to interfere considerably with visual function.

As our procedure for tissue-engineered oral mucosal epithelial sheets for ocular transplantation is relatively new, it is too early for long-term results regarding the longevity of the improved corrected vision. We can, however, report that in our hands, cultivated autologous oral mucosal epithelial sheet transplantation is a safe procedure that led to no severe postoperative complications. Furthermore, our autologous transplantation provides rapid epithelial covering without the threat of an immunologic rejection. It also provides for a much-improved prognosis of ocular surface reconstruction compared with the conventional procedure. In fact, this study improved the surgical results of two cases failed by the conventional epithelial transplantation, indicating the superior advantages of our new procedure. Analysis of the biologic aspects of tissue-engineered oral mucosal epithelium sheets will lead to further improvements. Our autologous transplantation procedure may require short-term, postoperative immune suppression for the reduction of postoperative inflammation and control primary diseases, however, it can be safely performed even on very young patients. Cultivated autologous oral mucosal epithelial sheet transplantation constitutes a promising treatment in patients with severe ocular surface disorders.

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REPORTING VISUAL ACUITIES

The AJO encourages authors to report the visual acuity in the manuscript using the same nomenclature that was used in gathering the data provided they were recorded in one of the methods listed here. This table of equivalent visual acuities is provided to the readers as an aid to interpret visual acuity findings in familiar units.

Table of Equivalent Visual Acuity Measurements

Snellen Visual Acuities				
4 Meters	6 Meters	20 feet	Decimal Fraction	LogMar
4/40	6/60	20/200	0.10	+1.0
4/32	6/48	20/160	0.125	+0.9
4/25	6/38	20/125	0.16	+0.8
4/20	6/30	20/100	0.20	+0.7
4/16	6/24	20/80	0.25	+0.6
4/12.6	6/20	20/63	0.32	+0.5
4/10	6/15	20/50	0.40	+0.4
4/8	6/12	20/40	0.50	+0.3
4/6.3	6/10	20/32	0.63	+0.2
4/5	6/7.5	20/25	0.80	+0.1
4/4	6/6	20/20	1.00	0.0
4/3.2	6/5	20/16	1.25	−0.1
4/2.5	6/3.75	20/12.5	1.60	−0.3
4/2	6/3	20/10	2.00	−0.3

From Ferris FL III, Kassoff A, Bresnick GH, Bailey I. New visual acuity charts for clinical research. Am J Ophthalmol 1982;94:91–96.

Biosketch

Tsutomu Inatomi, MD, PhD, a faculty member at the Department of Ophthalmology at the Kyoto Prefectural University of Medicine in Kyoto, Japan. He graduated and received clinical training and his PhD at the Kyoto Prefectural University of Medicine. He spent 3 years as a research fellow at Schepens Eye Research Institute in Boston. His current interests include treatment of ocular surface disorders using regenerative medical procedures.

Ocular Surface Reconstruction With Combination of Cultivated Autologous Oral Mucosal Epithelial Transplantation and Penetrating Keratoplasty

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• PURPOSE: To report an assessment of the two-step surgical combination of cultivated autologous oral mucosal epithelial transplantation (COMET) and penetrating keratoplasty (PKP) used to treat patients with severe limbal deficiency disorders, and to investigate the keratin expression patterns of transplanted surviving oral mucosal epithelium.

• DESIGN: Observational case series.

• METHODS: Two patients with Stevens-Johnson syndrome and chemical eye injury were treated by COMET followed, approximately six months later, by a PKP triple procedure. In the course of a mean follow-up period of 22.5 months, their clinical outcomes and the efficacy of this two-step surgical procedure were assessed. In addition, the keratin expression in corneal buttons excised during PKP were immunohistochemically examined to characterize the oral mucosal epithelium that survived ectopically on the cornea. In vivo laser confocal microscopy was used to investigate the structure of the epithelium on the corneal grafts.

• RESULTS: The ocular surfaces were successfully reconstructed with cultivated autologous oral mucosal epithelial sheets and PKP. No clinical complications, such as persistent epithelial defects, rejections, or recurrence of cicatrization, were encountered. Postoperative best-corrected visual acuity was 20/125 in one patient and 20/100 in the other. The surviving oral mucosal epithelium, distinguished by its fluorescence pattern, consisted of an irregular, nonkeratinized, stratified epithelium without goblet cells. Immunohistochemical study demonstrated that K3, but not K12, was expressed in the transplanted cultivated oral mucosal epithelium that was similar to oral mucosal tissue. In vivo, the epithelial structure and cell density in the basal cell layer of the corneal grafts were similar to normal cornea.

• CONCLUSIONS: This study presents a two-step surgical approach to treat severely scarred ocular surfaces by means of a combination of COMET and PKP. Clinical outcomes suggest that this treatment may be beneficial for the maintenance of the reconstructed ocular surface by providing oral mucosal epithelium around the corneal graft. (Am J Ophthalmol 2006;142:757–764. © 2006 by Elsevier Inc. All rights reserved.)

BECAUSE SEVERE STEM CELL DEFICIENCY IS SOMETIMES accompanied by severe corneal stromal opacity and/or corneal endothelial dysfunction, most patients require penetrating keratoplasty (PKP) for visual rehabilitation. However, ocular surface reconstruction through corneal epithelial transplantation and PKP increases the risk for immunologic rejection and graft failure, and patients require long-term intensive immunosuppression and continuous care.[1,2]

Another clinical problem encountered in ocular surfaces reconstructed with PKP is the persistence of an epithelial defect after loss of the donor corneal epithelium. PKP without epithelial transplantation results in persistent epithelial defects as a result of the limited life span of the donor central corneal epithelium, especially in patients with limbal deficiency; the resultant graft-melting and conjunctival invasion severely compromises visual recovery. Therefore, to improve the clinical outcome and long-term

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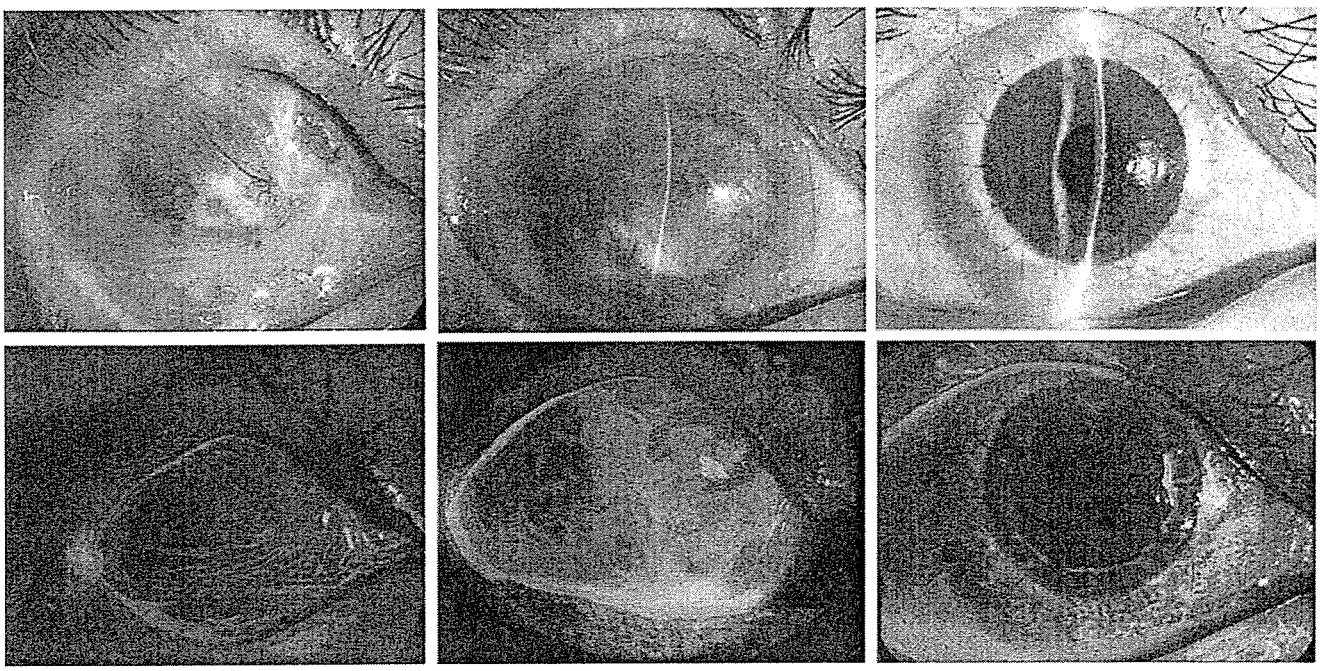

FIGURE 1. Clinical appearance before and after ocular surface reconstruction using cultivated autologous oral mucosal epithelial transplantation (COMET) and penetrating keratoplasty (PKP) in patient 1, a 70-year-old man with chemical injury. (Top left) Preoperatively, there is total conjunctivalization with severe scarring of both the cornea and conjunctiva. (Bottom left) Fluorescein staining. (Top center) Two months after initial surgery with COMET. (Bottom center) Uneven, hyperfluorescein staining pattern indicates survival of oral mucosal epithelium on ocular surface. (Top right) Status three months after PKP with cataract surgery. (Bottom right) Fluorescein staining demonstrated the slow invasion of oral mucosal epithelium surrounding the corneal graft.

prognosis of these patients, their reconstructed ocular surfaces must be provided with a more stable epithelial supply.

Pellegrini and associates[3] first reported the transplantation of cultivated corneal epithelium. Subsequent technical and surgical advances have made possible the grafting of cultivated corneal epithelial stem cell sheets.[4–8] Nakamura and associates[9] reported the successful transplantation of cultivated mucosal epithelial stem cell sheets derived from autologous cell sources. Autologous conjunctival epithelium[10,11] and nonocular (for example, oral mucosal) epithelium[12] have been used as a cell source for the cultivation of grafts to treat patients with bilateral ocular disorders. Because of its high proliferation potential, short cell-turnover time, and the safety of oral biopsy, oral mucosal epithelium has attracted attention as a cell source.[13,14] Initial clinical studies and midterm assessments of cultivated autologous oral mucosal epithelial transplantation (COMET) yielded favorable results from the perspective of ocular surface stabilization and visual recovery.[15–17] However, the cell biology and the longevity of surviving oral mucosal epithelium on the ocular surface require further investigation.

This study presents a two-step surgical strategy that uses a combination of COMET and PKP. The ocular surface was stable and the cornea remained transparent after the transplantation of cultivated oral mucosal epithelium when this two-step process is used. This surgical strategy reconstructs the ocular surface by transplanting a corneal graft that is surrounded by ectopically transplanted autologous oral mucosal epithelium just after the second PKP surgery, and the ectopically transplanted autologous oral mucosal epithelium may gradually cover the graft surface. This offers the potential for supplying mucosal epithelium for prolonged periods, and this high proliferation potential could possibly address the issue of wound healing. There is no direct evidence to date that oral mucosal epithelium would display a higher level of proliferation than ocular surface epithelium, but previous studies have demonstrated that oral mucosal epithelium has a high proliferation potential compared with epidermal cells.[13,14] On the basis of the condition of the oral mucosal epithelium, it is worth noting that this surgical concept and modality appear to have improved the clinical outcome of ocular surface disease that previously had a poor prognosis, although the follow-up period after PKP is relatively short.

METHODS

THIS STUDY WAS APPROVED BY THE INSTITUTIONAL REview Board for Human Studies of Kyoto Prefectural University of Medicine, and prior informed consent was obtained from all patients in accordance with the tenets of

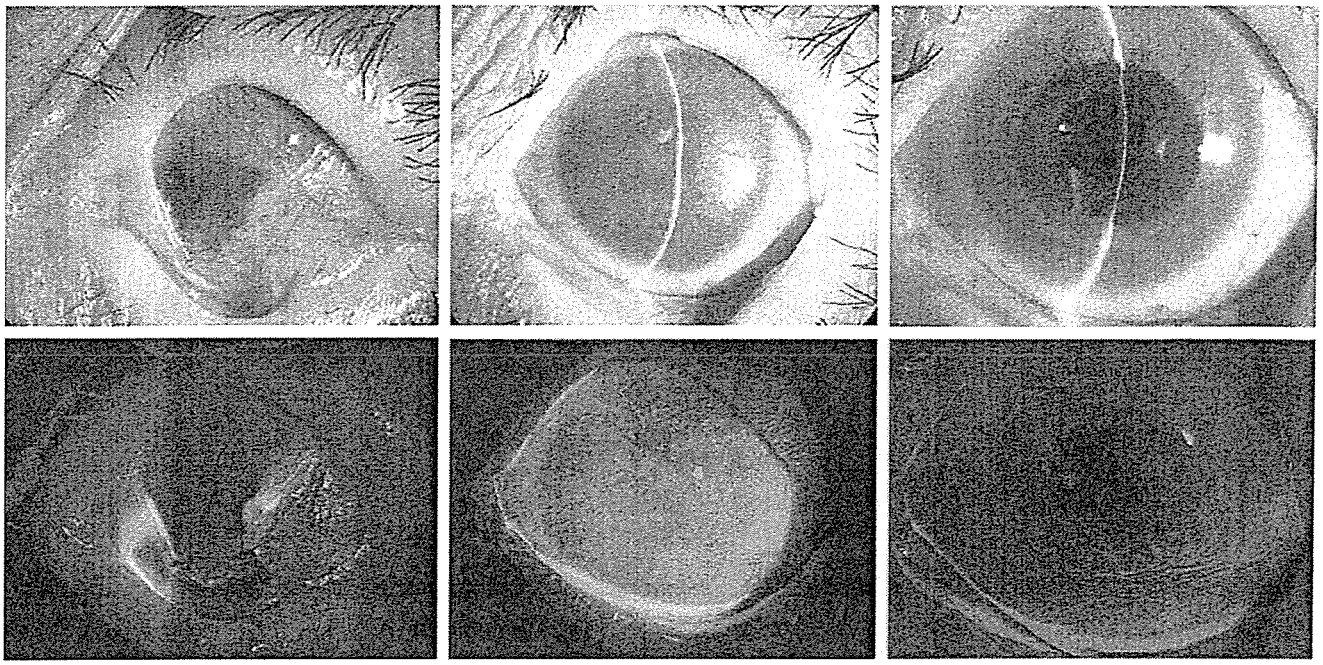

FIGURE 2. Clinical appearance before and after reconstruction using cultivated autologous oral mucosal epithelial transplantation (COMET) and penetrating keratoplasty (PKP) in patient 2, a 71-year-old man with Stevens-Johnson syndrome. (Top left) Preoperative total conjunctivalization with severe symblepharon and partial parakeratinization. (Bottom left) Fluorescein staining. (Top center) Two months after the initial surgery with COMET. (Bottom center) Fluorescein staining of surviving oral mucosal epithelium distinguishes between corneal and conjunctival epithelium. (Top right) Three months after PKP with cataract surgery. (Bottom right) Fluorescein staining demonstrated the presence of thicker oral mucosal epithelium surrounding the corneal graft.

the Declaration of Helsinki for research involving human subjects. This study involved two patients with bilateral total limbal deficiency; their ages were 70 and 71 years, respectively. The primary reason for their limbal deficiency and cicatrization was severe chemical injury and Stevens-Johnson syndrome. Both patients manifested severe destruction of the ocular surface; limbal deficiency was unequivocally diagnosed on the basis of the total replacement by scarred conjunctival tissue and the complete absence of the palisades of Vogt (Figures 1 and 2). Minimum reflex tearing was noted by slit-lamp examination and the Schirmer test, and there was sufficient meniscus height to maintain a wet mucous surface. Both patients presented severe scarring involving the full thickness of the cornea and restricted visibility of anterior chamber components. Patients 1 and 2 were followed for 26 and 19 months, respectively.

Human amniotic membrane (AM) was harvested at the time of elective caesarean section; preservation was at −80°C. Under sterile conditions, the membranes were deprived of their amniotic epithelium by two hours' incubation at 37°C with ethylenediamine tetraacetic acid (EDTA) 0.02% solution to loosen cell adhesion. This was followed by gentle scraping with a cell scraper.

The procedure for generating cultivated oral mucosal epithelial sheets has been reported by Nakamura and associates.[12,15] Under local anesthesia, 3 to 5 mm^2 oral mucosal biopsy specimens were obtained after proper treatment of the oral cavity. After removing submucosal connective tissues, small explants were immersed in phosphate-buffered (PBS) saline solution containing antibiotics (50 IU/ml penicillin-streptomycin and 5 μg/ml amphotericin B), incubated at 37°C for one hour with 1.2 IU dispase, and then treated with trypsin-EDTA 0.05% solution for 10 minutes at room temperature (RT) to separate the cells. The oral mucosal epithelium was then placed on denuded AM spread on the bottom of culture inserts and cocultured with mitomycin C–inactivated 3T3 fibroblasts. The culture was submerged in medium until cell confluence and then exposed to air by lowering the level of the medium for one to two days to promote epithelial differentiation. Cultures were incubated at 37°C in a 5% CO_2–95% air incubator; the medium was changed daily.

The initial surgical procedure for ocular surface reconstruction was as described in previous reports.[5,15] In brief, after a 360-degree conjunctival peritomy, conjunctivalized tissue on the corneal surface and thick, fibrotic subconjunctival tissues were removed. The subconjunctival spaces were treated with mitomycin C 0.04% for five minutes and then vigorously washed with saline solution. Then AM transplantation was carried out to reconstruct the conjunctival fornix. The preserved AM was placed with epithelial side up and then sutured with 10-0 nylon. After excising the AM covering the corneal surface, a

19-mm–diameter piece of cultivated autologous oral mucosal epithelial sheet was transplanted onto the corneal surface and sutured with 10-0 nylon. The integrity of the cultivated epithelium was confirmed by fluorescein staining at the end of surgery. Postoperatively, the transplanted epithelial sheet was protected with a medical-use contact lens.

PKP was performed five to six months after the initial COMET ocular surface reconstruction. PKP with cataract surgery was performed according to the usual procedures. In brief, a 7-mm–diameter trephination was performed on the host cornea, followed by continuous circular capsulorrhexis. The lens was removed by the regular phaco amelification and aspiration technique through the trephinated cornea. After inserting the intraocular lens, a 7.25-mm–diameter fresh donor cornea with epithelium was fastened with interrupted and continuous sutures. The corneal surface was then covered with a soft contact lens that was changed as appropriate during the follow-up period.

Immunohistochemical studies of keratin expression in the reconstructed ocular surface epithelium derived from cultivated oral mucosal epithelium were performed by using the previously described procedure.[12] Corneal buttons excised with a 7-mm–diameter trephine were examined at the time of the second surgery. Normal oral tissue was the control for immunohistochemical comparison studies; all tissues were stored at −80°C. Cryostat sections (7 μm in thickness) were placed on gelatin-coated slides, air dried, and rehydrated in PBS for 15 minutes at RT. The tissues were then incubated for 30 minutes at RT with bovine serum albumin 1% to block nonspecific bindings and further incubated (one hour, RT) with primary antibodies. Mouse monoclonal antibodies were used against keratin 1/10/4/13 (Novocastra, Newcastle, United Kingdom), keratin 3 (Progen, Heidelberg, Germany), and rabbit polyclonal antibodies against keratin 12 (Transgenic, Kumamoto, Japan). Control incubations were with appropriate normal mouse and rabbit IgG (Dako, Kyoto, Japan) at the same concentration as the primary antibody. After staining with the primary antibody, sections were incubated (one hour, RT) with the appropriate secondary antibodies; we used fluorescein isothiocyanate–conjugated donkey anti-mouse IgG (Jackson ImmunoResearch, West Grove, Pennsylvania, USA) and fluorescein isothiocyanate–conjugated donkey anti-rabbit IgG (Vector Laboratories, Burlingame, California, USA). After several washes with PBS, the sections were coverslipped with antifading mounting medium containing propidium iodide (Vectashield; Vector Laboratories) and examined under a confocal microscope (Fluoview; Olympus, Tokyo, Japan).

After more than one year of regular follow-up, an in vivo laser confocal microscope (Heidelberg Retinal Tomograph II/Rostock cornea module [HRT II]; Heidelberg Engineering, Heidelberg, Germany) was used for in vivo morphologic study of the reconstructed corneal epithelium on the corneal graft.[18] Confocal images in central regions were scanned from the apical layer to the basal epithelium. The density of the in vivo epithelium was measured by a computerized analysis system provided with the HRT II instrument.

RESULTS

ORAL MUCOSAL TISSUES WERE SAFELY EXCISED WITHOUT any complications. Approximately 1×10^5 oral mucosal epithelial cells were seeded onto the denuded AM and cultured for five to eight days until they reached confluence covering the entire AM. By two-week cultivation and air lifting, mature oral mucosal epithelium sheets that consisted of 5 to 6 cell layers were generated. Histologic examination confirmed that the sheets were comprised of well-differentiated stratified epithelium similar to that of the in vivo cornea; they consisted of a basal layer formed by cuboidal cells, several suprabasal cell layers, and flat apical cell layers. The condition of the cultivated epithelial sheet was confirmed by fluorescein staining at the end of the transplantation procedure. Both cases showed that the cultivated epithelial sheets were well stratified and without epithelial defect or any remarkable surface damage.

Patient 1 was a 70-year-old man who had experienced alkali burns to both eyes when he was 30 years old. Although history of previous surgeries was unavailable, slit-lamp examination showed round scarring in the peripheral cornea suggestive of earlier PKP. His right eye, chosen for ocular surface reconstruction, showed complete conjunctivalization on the corneal surface with extensive scarring and symblepharon formations (Figure 1). The intraocular status was unascertainable, yet ultrasound examination returned no abnormal vitreoretinal findings. His best-corrected visual acuity (BCVA) of the right eye was hand motion. On October 17, 2003, he underwent COMET and AM transplantation after the removal of scar tissue from both the cornea and subconjunctival space. Survival of the entire oral mucosal epithelium was confirmed on the second postoperative day, and it gradually covered the entire ocular surface. His visual acuity remained unchanged after the initial surgery. After the initial surgery, the reconstructed ocular surface showed uneven and irregular fluorescein staining absent of any epithelial defects (Figure 1). He experienced no recurrence of cicatrization or prolonged inflammation after the first operation. It is notable that the ocular surface before PKP was stable and uniform without inflammation. Because intraoperative observation showed the existence of a previous small PKP, PKP was selected to remove the corneal scar rather than lamellar keratoplasty. The second step, PKP combined with cataract surgery, was performed six months after the initial surgery; the graft had remained clear without any epithelial defect or rejection. There was minimal neovascularization along the sutures, but not in the corneal graft. A slow ingrowth of trans-

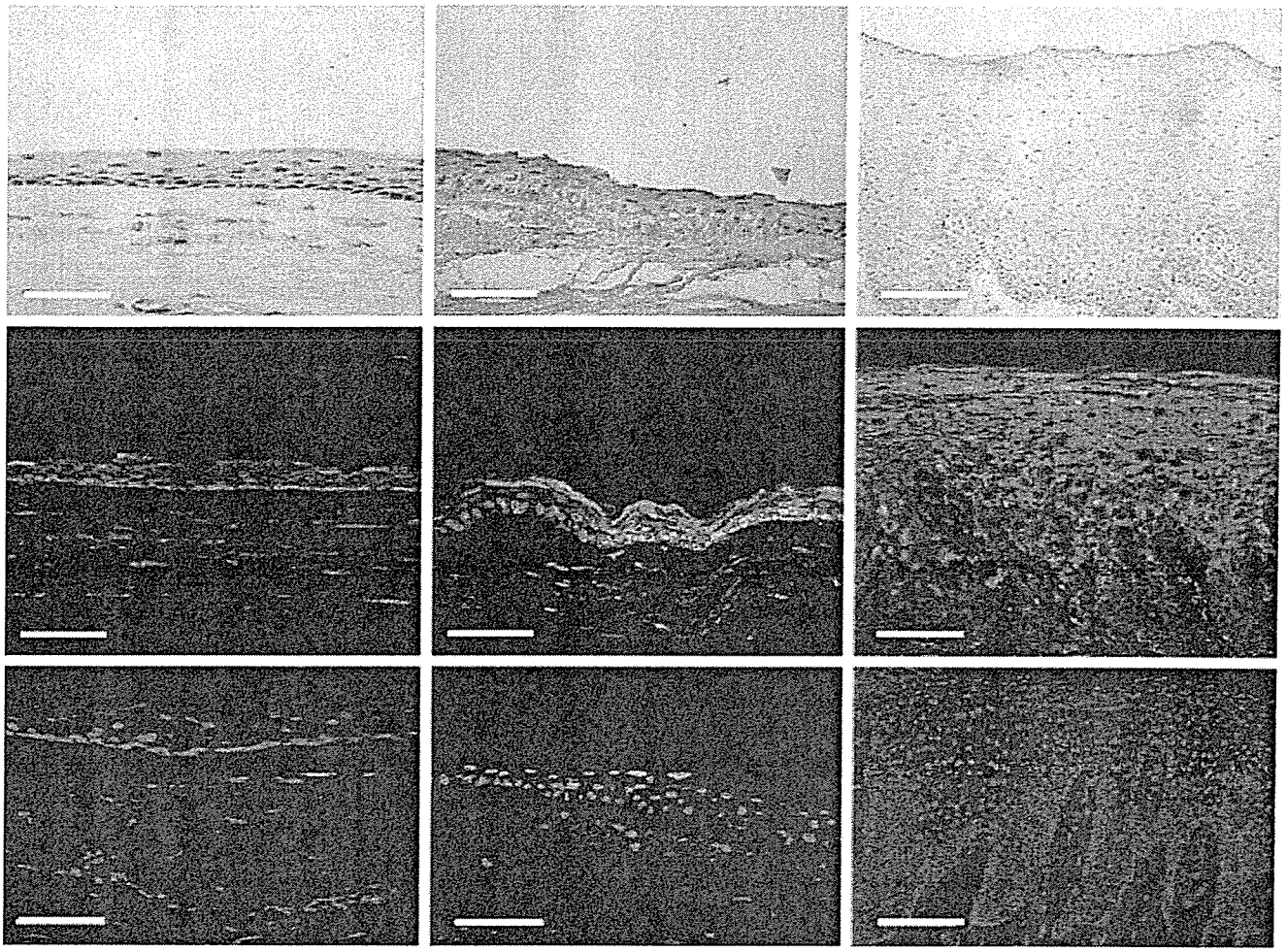

FIGURE 3. Immunohistological appearance of keratin 3 and 12 expressions in a cultivated oral mucosal epithelium sheet, surviving cultivated oral mucosal epithelium on the corneal button (subsequently resected at the time of penetrating keratoplasty [PKP]), and normal oral mucosal tissue. (Top left) Hematoxylin and eosin staining of a cultivated oral mucosal epithelial sheet from patient 1. (Second row, left) K3 expression in excised corneal button from patient 1. (Bottom left) There is no K12 expression in the excised corneal button from patient 1. (Top middle) Hematoxylin and eosin staining of cultivated oral mucosal epithelial sheet from patient 2. (Middle row, middle) K3 expression in excised corneal button from patient 2. (Bottom middle) K12 expression in excised corneal button from patient 2. (Top right) Hematoxylin and eosin staining of normal oral mucosal epithelium. (Middle row, right) K3 expression in normal oral mucosal epithelium. (Bottom right) Normal oral mucosal epithelium does not express K12. (Left and middle) Scale bars = 100 μm. (Right) Scale bar = 200 μm.

planted oral epithelium from the limbus was observed in the course of long-term follow-up (Figure 1). His BCVA improved to 20/100 and remained stable without reduction. Although his intraocular pressure (IOP) was occasionally high, he did not require glaucoma surgery. The occasional increase in IOP was managed by the topical application of carteolol hydrochloride 0.02% twice daily and latanoprost 0.05% once daily. Carbonic anhydrase inhibitor was also used to reduce IOP if the topical medication was not enough; however, no glaucoma surgery was required to control IOP.

Patient 2 was a 71-year-old man with no history of previous surgical treatment who had acquired Stevens-Johnson syndrome in his 40s. As shown in Figure 2, this patient had total conjunctivalization and severe scarring. He manifested minimal tear secretion and partial parakeratinization. Preoperatively, his visual acuity was hand movement. COMET was performed on this patient on May 26, 2004. There was an early epithelial defect in the center region during the two weeks after surgery; however, it healed without corneal melting or conjunctival invasion. His visual acuity remained unchanged after the initial operation. The second step, PKP with cataract surgery but not lamellar keratoplasty, was performed 5.5 months later by means of the standard procedure from the point of early visual rehabilitation. Subsequently, his BCVA improved to 20/125. He developed no postoperative complications except for a total corneal epithelial defect that originated

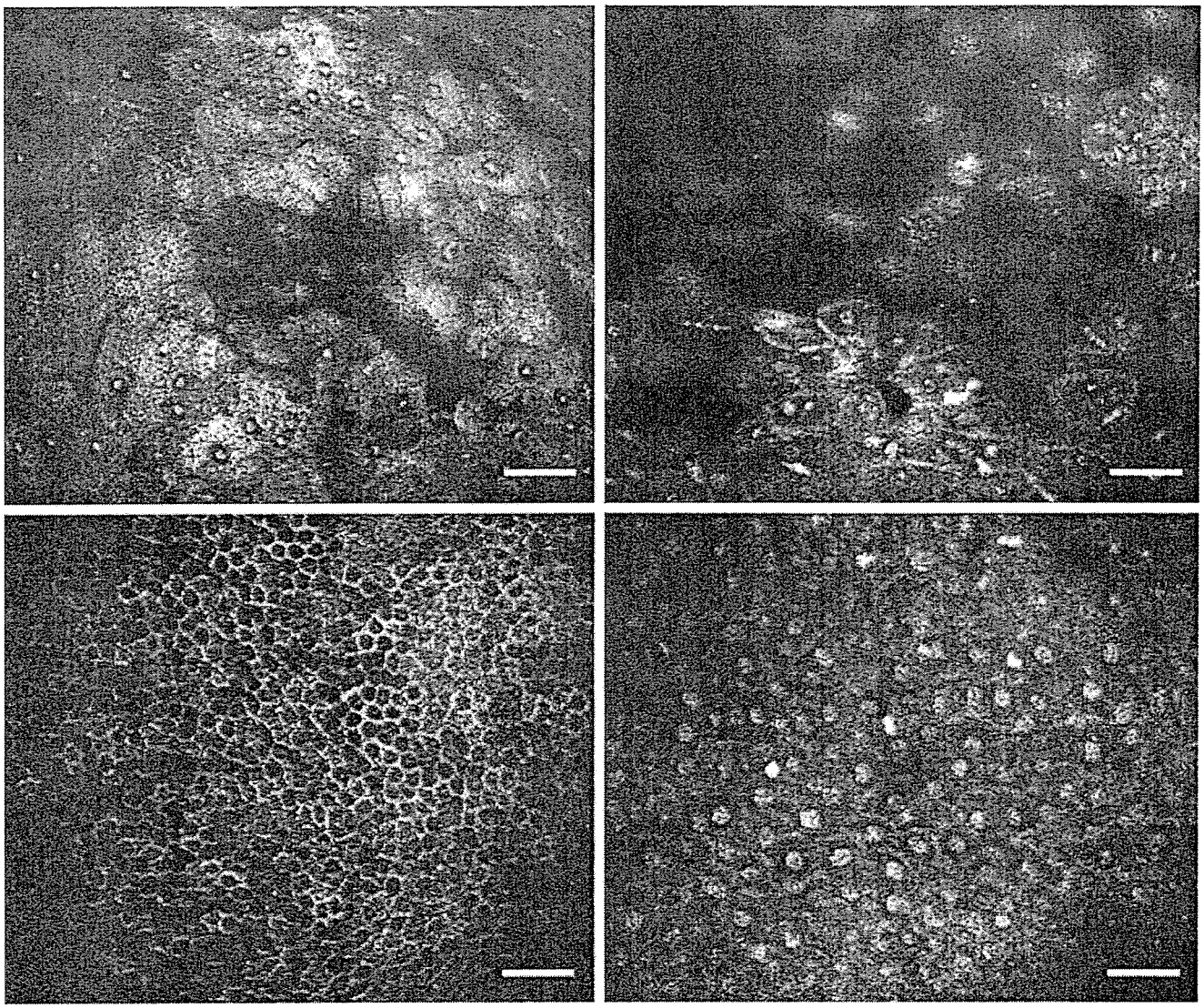

FIGURE 4. In vivo confocal micrographs demonstrate the appearance of the epithelium in the region of the transplanted central corneal surface. (Top left) Apical surface in patient 1. (Top right) Apical surface in patient 2. (Bottom left) Basal cell layers in patient 1. (Bottom right) Basal cell layers in patient 2. Note that the cell shape and density in each region are similar to normal cornea. Scale bars = 50 μm.

in the donor cornea after the medical contact lens fell off. However, the defect was gradually reepithelialized from the surrounding oral mucosal epithelium after rewear of the medical contact lens.

Immunohistochemical analysis was performed on the surviving transplanted cultivated oral mucosal epithelium on the cornea excised during PKP (Figure 3). Both patients demonstrated nonkeratinized stratified epithelium on the AM covering the cornea. Notably, in different regions, the stratified epithelium consisted of three to 10 layers; this finding was consistent with the results of slit-lamp examination. None of the specimens contained goblet cells. Immunohistochemistry confirmed the presence of K4 and K13; these keratins are specific for mucosal epithelium (data not shown). The expression of K1, which is specific for keratinized epithelium, was not detected (data not shown). As expected, K3 was expressed in surviving epithelium on the cornea as well as in oral mucosal epithelium. Conversely, K12, which is specific for corneal epithelium, was not expressed in the surviving epithelium, except for faint, occasional staining in the apical region.

We used the HRT II instrument for in vivo laser confocal scanning to study the histologic structure of the transplanted epithelium (Figure 4). The presence of a large, flat epithelium with small cell nuclei in the apical surface was noted in both patients; this is consistent with the normal corneal surface. The average cell density in the apical layer of the corneal graft was 840 ± 295 cells/mm^2 (mean ± SD) and not markedly different from a normal cornea.[18] The basal cells were smaller, denser, and aligned

in regular fashion, this also is similar to the normal corneal structure. The density of basal cells in the two patients was 8075.3 and 1492.0 cells/mm^2, respectively; in patient 1 it was within the range reported for normal central cornea (8996 ± 1532 cells/mm^2), whereas in patient 2 it was below the normal range.[18]

DISCUSSION

THIS STUDY PRESENTS A TWO-STEP SURGICAL APPROACH to treat patients with severe limbal deficiency disorder and corneal opacity. It consists of a combination of COMET and the conventional PKP triple-procedure. The two patients were followed for a mean of 22 months and encountered no immunologic rejection or persistent epithelial defect, common critical complications after combined surgical treatment consisting of corneal epithelial transplantation and PKP.

Cultivated autologous corneal epithelial transplantation that used AM was first introduced by Tsai and associates[4] and Koizumi and associates.[5] This tissue-engineered procedure promotes a strategy for reconstructing the corneal surface with autologous oral mucosal epithelium. This histologic study of the central cornea of two patients documents that transplanted cultivated oral mucosal epithelium on the corneal surface remained intact for at least the first six months after transplantation. Immunohistochemically, the surviving transplanted cultivated oral mucosal epithelium on the cornea was positive for K3 and K4 (data not shown) and negative for K10 (data not shown) and K12, indicating that it was neither corneal nor conjunctival. Rather, it resembled cultivated oral mucosal epithelium grown on AM. Thus, the intrinsic characteristics of the ectopically transplanted epithelium did not change. This finding coincides with observations made when cultivated oral mucosal epithelial sheets were transplanted onto rabbit eyes.[12] Because epithelial differentiation largely depends on the substrate, transplanted cultivated oral mucosal epithelial sheets do not resemble the in vivo oral mucosal epithelium, probably because of modifications induced by the external environment—that is, the corneal stroma or AM. The absence of neovascularization into the cornea after the grafting of oral mucosal epithelium may also be attributable to interaction with the corneal stroma. Studies are currently underway to elucidate biologic factors, such as mucin expression by surviving oral epithelium, to gain an understanding from the perspective of corneal function.

To improve the success rate of ocular surface reconstruction with PKP, allogenic recognition by the host immune system must be minimized. Therefore, limbal transplantation was avoided and a two-step approach was used instead. Tsubota and associates[19] demonstrated better graft survival when a two-step procedure was used to treat severe ocular surface disorders. The survival rate of limbal transplants and PKP grafts after combined surgery is relatively poor.[1,2,19] Because the limbal region contains allogenic antigens such as antigen-presenting cells and major histocompatibility complex class 2 molecules, allogenic limbal transplantation may be inappropriate in patients with severe limbal deficiency disorders. Although the findings of this study must be considered preliminary, they suggest that the mucosal epithelium covering the cornea, because it was derived from autologous oral mucosal epithelium, is not subject to allosensitization. Therefore, the results of this study indicate that this two-step procedure involves a risk for endothelial rejection that is no greater than that encountered with conventional PKP.

This two-step procedure features another improvement: the continuous, prolonged supply of epithelium, which compensates for the limited survival of corneal epithelium on the central corneal graft. However, although no epithelial defect was observed during the 22-month follow-up period, additional long-term observations are necessary to determine whether oral mucosal epithelial cells will offer continuous replacement on the transplanted cornea. Patients with limbal stem cell–deficient eyes often manifest persistent epithelial defects on their grafts after PKP. The proliferation potential of conjunctival epithelium is relatively low, and this may partly explain the persistence of the epithelial defects. Oral mucosal epithelium is thought to be less well differentiated, and this may be an advantage in terms of short cell turnover time and a quicker wound-healing response after transplantation.[13,14] However, no comparison of the relative rates in epithelial healing for ocular surface epithelial cells compared with oral epithelial cells was attempted in this study. Hayashida and associates[20] used a rabbit model to demonstrate that in vivo and in cultivated sheets, p63- and integrin 1–positive cells manifested the higher proliferation characteristics of oral mucosal epithelial cells. Inatomi and associates[17] previously reported positive midterm results in patients who had undergone ocular surface reconstruction by COMET. This in vivo laser confocal microscopic study demonstrated that the stratified epithelium existed in the central zone of the two patients. The shape of apical cells and size and density of the basal cells were similar to normal cornea, suggesting the maintenance of a well-differentiated structure of graft after the ocular surface reconstruction. At present, it is unclear whether the epithelium examined in this study was transplanted allogenic corneal epithelium or regenerated epithelium derived from autologous cultivated oral mucosal epithelium on the peripheral cornea.

The results of experimental and clinical studies suggest that COMET is a promising and advantageous alternative to mucosal epithelium transplants for ocular surface reconstruction. This study documented the survival of ectopically transplanted oral mucosal epithelium and showed that the transplantation of autologous oral mucosal stem cells to donor corneal grafts avoids common epithelial complications. At present, the long-term survival of both

the transplanted oral mucosal epithelia and allogenic corneal grafts in this study continue to be monitored.

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Biosketch

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Functional Visual Acuity in Stevens-Johnson Syndrome

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• PURPOSE: To evaluate the correlation of functional visual acuity (FVA) measurement with ocular surface findings in patients with Stevens-Johnson syndrome (SJS).
• DESIGN: Prospective comparative study.
• METHODS: Sixty-nine eyes of 38 patients with chronic SJS assessed at the Tokyo Dental College, Tokyo Medical Center, and the Kyoto Prefectural University of Medicine, Department of Ophthalmology, Kyoto, Japan, were studied. Twenty eyes of 10 normal subjects and 40 eyes of 20 patients with Sjögren syndrome (SS) were also studied. Conventional Landolt visual acuity (VA) and FVA examinations and slit-lamp examinations were performed. FVA was measured continuously by the FVA measurement system during a 30-second blink-free period in one eye. The visual maintenance ratio (VMR) was calculated as follows: VMR = [(2.7 − FVA)/(2.7 − baseline VA)], where logarithm of minimal angle of resolution values of FVA were entered into the formula and 2.7 represented the lowest visual acuity in this series. Slit-lamp examinations, Schirmer test, and fluorescein vital stainings were also performed in all subjects.
• RESULTS: VMR was markedly lower in patients with SJS compared with patients with SS and controls. FVA values showed a relation with the presence of corneal opacity and vascularization.
• CONCLUSIONS: The FVA measurement system is not only a useful tool in the evaluation of dynamic VA changes, but also reflects the ocular surface clinical findings in SJS. (Am J Ophthalmol 2006;142: 917–922. © 2006 by Elsevier Inc. All rights reserved.)

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STEVENS-JOHNSON SYNDROME (SJS) IS A SEVERE OCULAR surface disease that may be associated with poor visual prognosis.[1–3] Symblepharon, adhesive occlusion of the lacrimal puncta, and corneal opacification with conjunctivalization are often observed in the chronic stages of the disease.[4] Severe dry eye due to the absence of reflex tearing is another major problem in such patients,[5] leading to worsening of ocular-surface health.[2,6,7] It has been reported that a stable tear film over the corneal surface is essential for clear visual imaging and that irregular corneal surface resulting from dry eyes is associated with a poor quality of vision.[8–12]

The functional visual acuity (FVA) measurement system is thought to reflect the dynamic changes in visual acuity (VA) reflecting the status of vision related to daily activities. With the FVA measurement system, dynamic visual changes are continuously measured under a 30-second blink-free period in one eye. We previously reported that FVA was worse in patients with Sjögren syndrome (SS) and non-SS dry eyes compared with controls.[13] In this report, we performed FVA measurements in patients with SJS and compared the results with normal, healthy subjects and in patients with SS. We also studied the correlation between FVA measurements and the clinical findings of the ocular surface status in patients with SJS.

METHODS

SIXTY-NINE EYES OF 38 CONSECUTIVE PATIENTS (20 MALE and 18 female patients; mean ± SD age 42.6 ± 15.4 years; range, eight to 74 years) with SJS assessed at the Cornea Subspecialty Outpatient Clinic of the Department of Ophthalmology of Tokyo Dental College, Tokyo Medical Center, and Kyoto Prefectual University, Japan, were studied. This research followed the tenets of the Declaration of Helsinki. Informed content was obtained from all subjects. Ethic committee approvals were obtained at each center for this study.

The diagnosis of SJS was based on the history of the presence of cryptogenic fever and acute inflammation of

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TABLE 1. Grading of Severity Scores of Ocular Surface Clinical Findings in Patients With Stevens-Johnson Syndrome and Sjögren Syndrome

Finding	Score			
	0	1	2	3
Trichiasis	None	<1/4 of upper and lower lid	≥1/4, <1/2 of upper and lower lid	≥1/2 of upper and lower lid
Symblepharon	None	Within conjunctival sac	<1/2 of cornea	≥1/2 of cornea
MGD				
Secretion	Meibum clear	Yellow cloudy	Granular, cheesy	Not expressible
MCJ	Normal	<1 mm behind NMCJ	≥1, <2 mm behind NMCJ	≥2 mm behind NMCJ
Conjunctivalization	None	<1/4 of cornea	≥1/4, <1/2 of cornea	≥1/2 of cornea
Central corneal opacity	None	Pupillary area visible	Lens hardly visible	Pupillary area not visible
Corneal vessels	None	Peripheral	At pupillary edge	Over the pupil
Fluorescein staining (points)	0	1~2	3~5	6~9

MGD = meibomian gland dysfunction; MCJ = mucocutaneous junction; NMCJ = normal mucocutaneous junction.

mucosal membranes after receiving antibiotic or anti-inflammatory drugs, and on the presence of the chronic ocular surface complications such as symblepharon, entropion, trichiasis, xerophthalmia, and corneal vascularization.[13–16] Twenty eyes of 10 healthy normal subjects (two male and eight female subjects; mean age, 42.5 ± 12.9 years; range, 25 to 70 years) without dry eyes and dry eye symptoms and 40 eyes of 20 patients with SS (two male and 18 female subjects; mean ± SD age 52.6 ± 15.4 years; range, 19 to 80 years) who were diagnosed according to Fox criteria were also investigated in this multicenter study.[17] None of the patients or control subjects had any other systemic or ocular diseases, history of ocular surgery within six months, or history of ocular cicatricial pemphigoid, chemical, thermal, or radiation injury that would have adverse ocular surface effects. Patients with SJS with a baseline conventional best-corrected Landolt VA score of less than 20/2000 as a result of cataract, ocular surface keratinization, glaucoma, or posterior segment disease were excluded from this study.

All study subjects underwent slit-lamp examinations including assessment of the status of eyelids, presence of meibomian gland disease, conjunctivalization of the cornea, presence of corneal opacity, and assessment of corneal vessels. A severity grade was assigned for each clinical finding (Table 1). The grading of Bron and associates[18] was used for the classification of mucocutaneous junction and meibum changes. The grading and ocular surface tests were performed under the same protocol discussed and accepted before the initiation of the study by the researchers of all contributing study centers.

The standard Schirmer test without topical anesthesia was performed. The standardized strips of filter paper (Alcon Inc, Fort Worth, Texas, USA) were placed in the lateral canthus away from the cornea and left in place for five minutes with the eyes closed. Readings were reported in millimeters of wetting for five minutes. A 2-μl volume of fluorescein dye 1% was instilled in the conjunctival sac by a micropipette. The minimum score for corneal fluorescein staining was zero points and the maximum score was nine points.[19] A vital staining severity grading was also assigned in this study (Table 1).

The FVA measurement system (Nidek, Tokyo, Japan) was used to examine the timewise change in continuous VA (Figure 1). The device is made up of three parts: a hard disk, a monitor, and a joystick. The Landolt optotypes are presented on the monitor, and their sizes change depend-

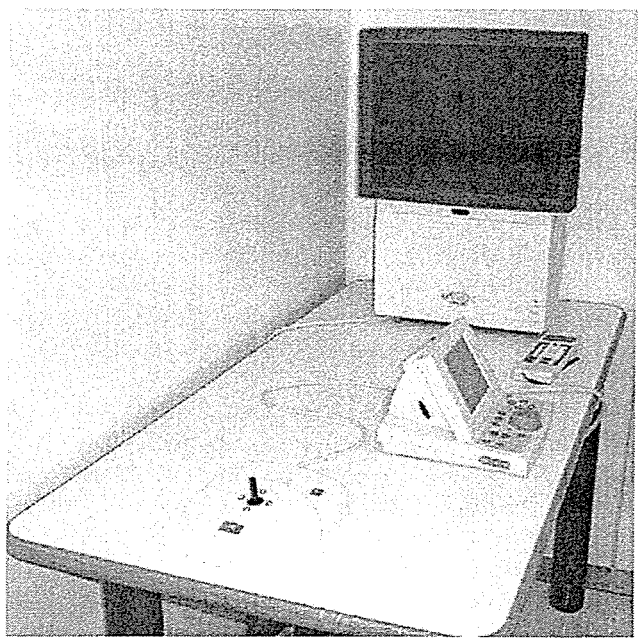

FIGURE 1. Functional visual acuity (VA) measurement system, which measures dynamic change in VA.

ing on the correctness of the responses. In brief, the optotypes are displayed automatically, starting with smaller ones. If the responses are incorrect, larger optotypes are presented automatically. When there was no response within the set display times, the answer was taken to be an error and the optotype automatically enlarged.

VA is continuously measured from the baseline best-corrected Landolt VA. The FVA measurement system can measure VA from 400/200 to 20/2000. The measurements were begun from the baseline established best-corrected Landolt conventional VA in each subject. Optotypes were presented at a distance of 2.5 m in patients with a baseline best-corrected VA ≥20/200. In subjects with a best-corrected VA <20/200, the testing distance was set at 1 m. The testing distance information is entered into the computer database so that each Landolt optotype presented on the monitor subtended an equivalent angle to the optotype of the same VA level presented from 5 m during the conventional Landolt VA testing. The presentation time of an optotype was adjusted at one second, and optotypes changed automatically within the previously set presentation time frame.

At the FVA measurement, one drop of topical anesthesia (oxybuprocaine chloride 0.4%) was administered 15 minutes before the examination to minimize discomfort and prevent reflex tearing and blinking. The upper eyelids of patients with SJS were elevated gently to allow continuous testing for 30 seconds. Patients delineated the orientation of the automatically presented Landolt rings by handling the joystick from the baseline best-corrected VA from the start. VA at 10, 20, and 30 seconds were checked as records of FVA and compared between patients with SJS, patients with SS, and control subjects. The FVA measurement system reported in this study is a new advanced version compared with the system previously reported by us, which allowed testing at 1.1 m only where the presented optotypes within the system subtended equivalent angles to the optotypes of the same VA level presented from 5 m. The previous device could not measure VA scores less than 8/200 and was also designed for clinical settings without enough distance/space to carry out vision measurements from 5 m. The current system not only allows functional VA testing at different distances, but also assesses VA in low-vision patients with VA scores ≥20/2000.

The baseline conventional best Landolt visual acuities, which were also the starting point for FVA measurements, were 20/20 or above in all patients with SS and normal control subjects. The baseline conventional Landolt visual acuities were below 20/20 in patients with SJS. To allow comparisons of timewise FVA changes between patients with SJS, patients with SS, and normal subjects, logarithm of minimal angle of resolution (logMAR) values of the FVA scores at 10, 20, and 30 seconds were divided by the logMAR baseline VA score. The lowest logMAR VA score was set at 2.7 for calculation of the FVA ratio: (2.7 − FVA at 10, 20, or 30 seconds)/(2.7 − baseline VA). The ratio allowed comparison of FVA at the testing points of 10, 20, and 30 seconds between the three groups.

A *t* test was performed for the comparison of FVA ratio scores at 10, 20, and 30 seconds of testing among patients with SJS and SS and normal controls subjects, as well as comparison of visual maintenance ratio (VMR). The relation between clinical ocular surface findings and FVA was investigated by multiple linear regression analysis. A probability level of $P < .05$ was considered statistically significant. SPSS software (SPSS Inc, Chicago, Illinois, USA) was used for statistical analysis.

RESULTS

THE MEAN SCHIRMER TEST VALUES WERE 10.3 ± 9.6 MM IN patients with SJS, 4.38 ± 3.66 mm in patients with SS, and 14.2 ± 9.4 mm in healthy control subjects. The Schirmer test values were higher in patients with SJS as compared with patients with SS; 56.9% of the patients with SJS had Schirmer test values >5 mm.

The average logMAR FVA showed a timewise decrease during testing in patients with SJS and SS and in normal subjects. Figure 2 shows the results of dynamic changes of VA. The timewise change of FVA was similar in patients with SJS and patients with SS, and the timewise decline of FVA was greater in patients with SS and patients with SJS compared with normal subjects. Mean logMAR FVA scores were lower in eyes of patients with SS and patients with SJS at 10, 20, and 30 seconds. The mean baseline VA score was lower in eyes of patients with SJS compared with control subjects.

Table 2 shows changes of VMR at 10, 20, and 30 seconds. VMR of patients with SJS were significantly lower compared with the normal subjects and patients with SS at 10, 20, and 30 seconds ($P< .05$).

Figure 3 shows the average severity scores of clinical findings in patients with SS and SJS. The mean clinical severity scores for trichiasis, symblepharon, meibomian gland dysfunction, corneal conjunctivalization, corneal opacity, and corneal vessels were markedly higher in patients with SJS compared with patients with SS. A total of 65.0% of the patients with SJS had grade 1 to 3 central corneal opacities. A total of 49.3% of the patients with SJS had grade 2 to 3 central vessels involving the central corneal area. A total of 58.8% of the patients with SJS had grade 0 to 2 corneal conjunctivalization not involving the pupillary area. A total of 42.6% of the patients with SJS had milder grade 0 to 1 meibomian gland dysfunction in this study.

Table 3 shows the relation of FVA at 10 seconds and the average scores of seven items of clinical findings. Dependent variable was logMAR FVA at 10 seconds, and independent variables were seven items of clinical findings. The results of multiple linear regression analysis