

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 cicatrization 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 report¹⁴ 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

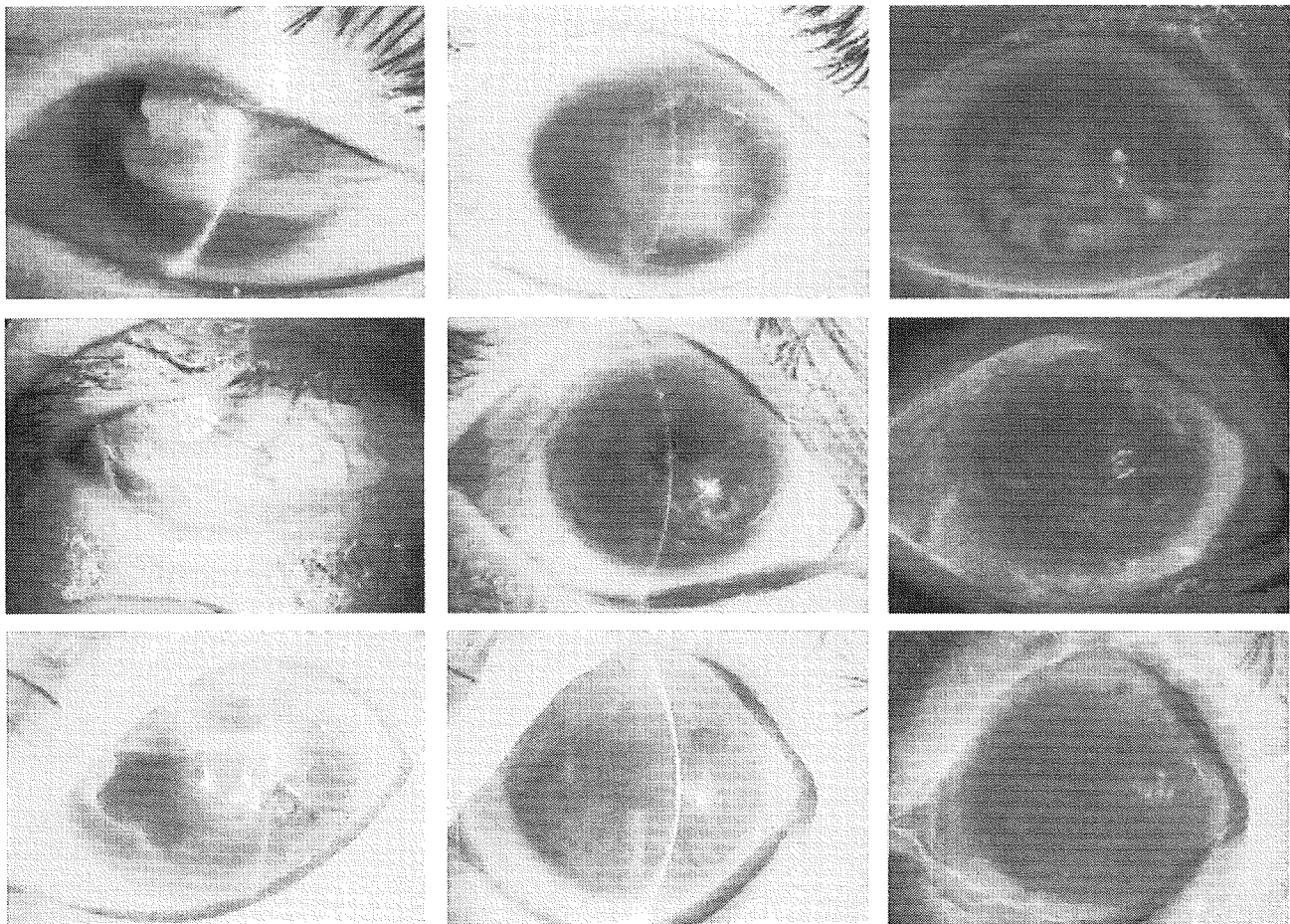


FIGURE 4. Slit-lamp photographs of three patients in the chronic phase of ocular surface disorders shown before and after ocular surface reconstruction using cultivated oral mucosal epithelial transplantation. Case 5 (14-year-old girl): chronic phase of SJS with severe symblepharon over the cornea. (Top 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-

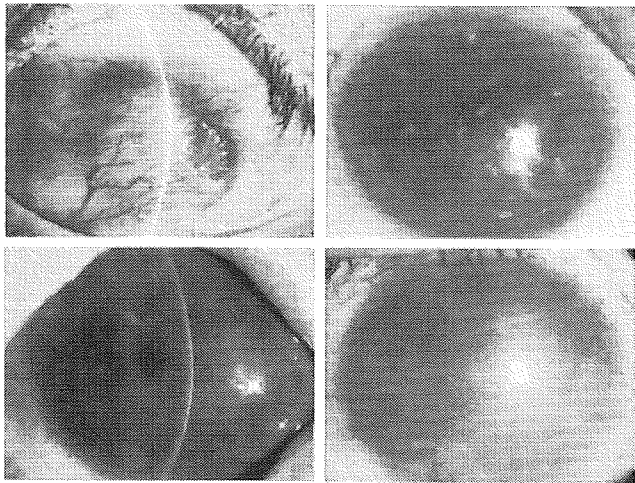


FIGURE 5. Slit-lamp photographs showing vascular formations after the transplantation of cultivated autologous oral mucosal epithelial sheets. (Top left) Preoperatively, most of the cornea manifested highly-vascularized conjunctivalization (case 3, grade 4). At the 1-year follow-up, vascular formations 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 associates¹⁶ demonstrated p63 and β 1-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			Decimal Fraction	LogMar
4 Meters	6 Meters	20 feet		
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

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