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TREATMENT OF SEVERE OCULAR-SURFACE DISORDERS WITH CORNEAL EPITHELIAL STEM-CELL TRANSPLANTATION

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ABSTRACT

Background Conditions that destroy the limbal area of the peripheral cornea, such as the Stevens–Johnson syndrome, ocular pemphigoid, and chemical and thermal injuries, can deplete stem cells of the corneal epithelium. The result is scarring and opacification of the normally clear cornea. Standard corneal transplantation cannot treat this form of functional blindness.

Methods We performed and evaluated 70 transplantations of corneal epithelial stem cells from cadaveric eyes into 43 eyes of 39 patients with severe ocular-surface disorders and limbal dysfunction. Medical treatment had failed in all patients. The patients had a mean preoperative visual acuity of 0.004 (only being able to count the number of fingers presented by the examiner) in the affected eyes, which satisfies the criteria for legal blindness in most countries. In 28 eyes, we also performed standard corneal transplantation. Stem-cell transplantations were performed as many as four times on 1 eye if the initial results were not satisfactory; 19 eyes had multiple transplantations. Patients were followed for at least one year after transplantation.

Results A mean of 1163 days after stem-cell transplantation, 22 of the 43 eyes (51 percent) had corneal epithelialization; of the 22 eyes, 7 eyes had corneal stromal edema and 15 eyes had clear corneas. Mean visual acuity improved from 0.004 to 0.02 (vision sufficient to distinguish the largest symbol on the visual-acuity chart from a distance of 1 m) ($P < 0.001$). The 15 eyes in which the cornea remained clear had a final mean visual acuity of 0.11 (the ability to distinguish the largest symbol from a distance of 5 m). Complications of the first transplantation included persistent defects in the corneal epithelium in 26 eyes, ocular hypertension in 16 eyes, and rejection of the corneal graft in 13 of 28 eyes. The epithelial defects eventually healed in all but two of the eyes.

Conclusions Transplantation of corneal epithelial stem cells can restore useful vision in some patients with severe ocular-surface disorders. (N Engl J Med 1999;340:1697-703.)

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THE cornea is a transparent, avascular tissue of the ocular surface whose integrity is vital for maintaining useful vision. The cornea is covered by stratified epithelium that is responsible for maintaining a smooth ocular surface as well as for providing a barrier against environmental stress. The most superficial cells are regularly shed from the surface of the eye and replaced by new cells that are ultimately provided by corneal epithelial stem cells located in the limbal area of the peripheral cornea (Fig. 1). Although stem cells of the corneal epithelium are not depleted under physiologic conditions, pathologic conditions such as chemical injuries, thermal injuries, the Stevens–Johnson syndrome, and ocular pemphigoid can cause destruction of the limbal epithelium. The loss of stem cells from the corneal epithelium leads to invasion of the cornea by vascularized conjunctival epithelium,¹ which causes functional blindness that cannot be treated by standard corneal transplantation.

In 1989, Kenyon and Tseng reported the successful transplantation of a limbal autograft obtained from the healthy eye in the treatment of severe unilateral ocular-surface disease.² For patients with bilateral disease, Tsai and Tseng,³ followed by Pfister⁴ and Tsubota et al.,⁵ reported the transplantation of corneal epithelial stem-cell allografts obtained from donor tissue. Modification of the technique by the use of amniotic membrane as a substrate replacement⁶ and eye drops containing autologous serum as a tear replacement⁷ has permitted new approaches to the treatment of even end-stage cicatricial diseases with complete loss of stem cells and tears.⁸ Such severe diseases have

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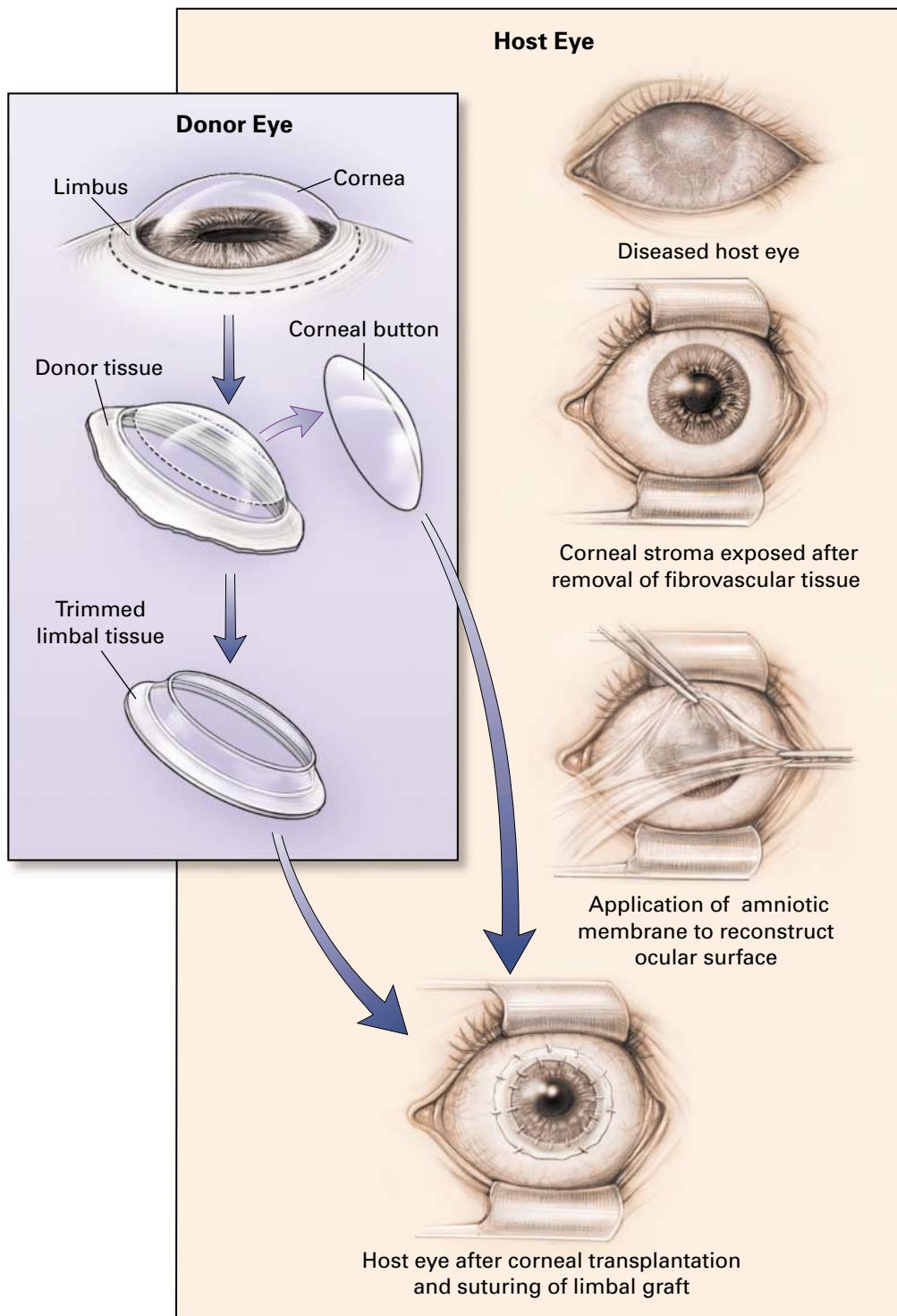


Figure 1. Corneal Epithelial Stem-Cell Transplantation.

First we cleared the host eye of all fibrovascular tissue invading the ocular surface in order to expose the corneal stroma. If the stroma was also involved, we excised either a lamellar or full-thickness segment, depending on the depth of involvement. We then applied amniotic membrane to reconstruct the ocular surface. Donor tissue was prepared from corneal scleral buttons (inset). We punched out and used the central clear cornea when simultaneous corneal transplantation was necessary. We manually dissected the remaining ring of tissue to remove excess tissue surrounding the limbal area. We then sutured the limbal graft onto the host eye with 10-0 nylon sutures.

been considered contraindications to surgery because of the extremely poor prognosis of patients treated with corneal transplantation.

Although preliminary reports of the transplantation of corneal epithelial stem cells are promising,^{3-5,9,10} a study reporting the eventual disappearance of donor-derived limbal cells in the recipient¹¹ has raised questions about the feasibility of this technique. We examined the long-term efficacy and complications of the transplantation of corneal epithelial stem cells as a treatment for severe disorders of the ocular surface.

METHODS

Patients

We transplanted stem-cell allografts in patients with severe ocular-surface disorders with limbal dysfunction.⁵ Dysfunction of the stem cells of the corneal epithelium was identified by the presence in the central cornea of goblet cells derived from the conjunctiva ("conjunctivalization"),¹ persistent epithelial defects (a lack of proliferation of the corneal epithelium), or completely keratinized epithelium (in which the tissue changes to resemble skin), accompanied by an absence of palisades of Vogt, a biologic marker of the location of stem cells in the limbal region.¹² In all patients, medical treatment, such as oral dapsone and corticosteroids, as well as topical corticosteroids and lubricants, had had limited success. Surgical treatment was chosen as a final resort. Written informed consent was obtained from all study participants. Only patients who were followed for more than 12 months after surgery were included in the study. The limbal transplantation was carried out in accordance with laws in Japan governing corneal transplantation.

The functional measure of vision used in the study was the visual acuity of the patients before and after surgery. Visual acuity is defined as the size of the smallest retinal image that can be seen by the patient; it is expressed in terms of the minimum visual angle (minutes of arc) projected onto the sensory retina. We adopted the decimal notation, which is equivalent to the reciprocal of the minimum visual angle; a visual acuity of 1.0 is thus 1/1 minute of arc, and 0.1 is 1/10 minutes of arc. The visual acuity of patients who could not distinguish visual-acuity charts at a distance of 1 m was assessed by asking whether they could count the number of fingers presented by the examiner. If they could not, visual acuity was recorded as the ability to see the hand movement of the examiner or, in worse cases, the ability to perceive light projected into the eye.

Classification of Disease

We classified all patients into two categories of primary stem-cell dysfunction according to the criteria of Puangricharern and Tseng¹: those with cicatricial keratoconjunctivitis associated with the Stevens-Johnson syndrome or ocular pemphigoid, and those with chemical or thermal injury (Table 1). Patients with limbal dysfunction due to other diseases were not included in the study.

Surgical Procedure

The surgical procedure was designed to remove all abnormal tissue invading the ocular surface and to provide corneal stem cells by transplantation of limbal allografts from cadaveric donors. We removed conjunctival or dermal epithelium covering the cornea and then dissected fibrous tissue and any existing symblepharon (adhesions between the conjunctiva of the lid and globe) that might have interfered with eye motility. When necessary, we performed additional penetrating or lamellar keratoplasty, with or without reconstruction of the anterior segment, and removed cataracts (Fig. 1 and 2).⁷

We used amniotic membranes as a replacement substrate when

TABLE 1. BASE-LINE CHARACTERISTICS OF THE 43 DISEASED EYES IN 39 PATIENTS, ACCORDING TO THE CAUSE OF DYSFUNCTION.*

CHARACTERISTIC	STEVENS-JOHNSON SYNDROME OR OCULAR PEMPHIGOID		CHEMICAL OR THERMAL INJURY	TOTAL
Patients				
No. of patients	25	14		39
Sex (no.)				
Male	13	13		26
Female	12	1		13
Age (yr)	52±24	43±20		49±23
Bilateral disease (no.)	4	0		4
Eyes				
No. of eyes	29	14		43
Mean visual acuity of the affected eye†	0.003	0.007		0.004
No. of limbal transplantations (no. of eyes)				
1	22	2		24
2	6	7		13
3	1	3		4
4	0	2		2
Mean no. of limbal transplantations	1.3	2.4		1.6
Corneal transplantation (no.)	15	13		28

*Plus-minus values are means ±SD.

†The mean visual acuity ranged from no light perception to 1.0.

underlying stromal tissue had been destroyed. Amniotic membranes are used in the reconstruction of the ocular surface to facilitate epithelialization and to reduce inflammation and scarring, which may compromise the success of limbal transplantation.⁷ Amniotic membrane was obtained in a sterile manner during cesarean sections after written consent had been obtained from the mother. We confirmed that maternal serum was negative for human immunodeficiency virus, hepatitis C virus, and hepatitis B virus. During limbal transplantation, we manually dissected the amniotic membrane from the chorion and then placed it on the ocular surface with the epithelial side facing outward. We then secured the membrane to the eye with eight 9-0 silk sutures^{7,13} (Fig. 1 and 2). We covered with the amniotic membrane as much of the ocular surface as possible, except for the palpebral portion of the conjunctiva under the eyelids.

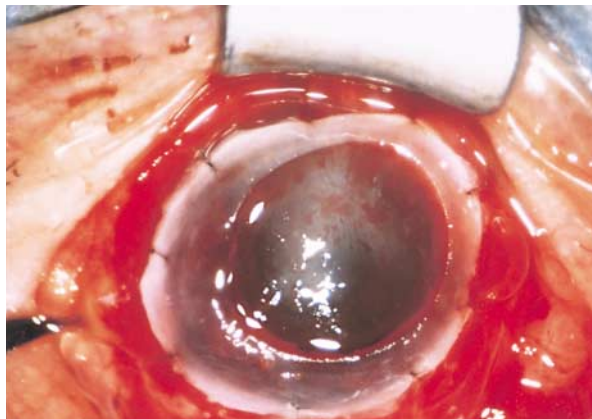
We prepared limbal tissue from eyes obtained from an eye bank. The mean (±SD) age of the donors was 57.2±13.0 years, and the mean period of tissue storage was 5.9±1.1 days. The corneal scleral buttons had been stored in Optisol GS medium (Chiron Vision, Irvine, Calif.), which is designed to preserve corneal tissue for up to 14 days, according to the routine procedure of the eye bank. We dissected and removed as much as possible of the stromal portion of the limbus, leaving thin, ring-shaped limbal tissue containing corneal epithelial stem cells (Fig. 1). We sutured this segment of limbal tissue to the original limbal area with 10-0 nylon sutures.

Postoperative Care

Patients received systemic immunosuppression, because limbal tissue is believed to be more prone to tissue rejection than are conventional corneal transplants. Treatment with oral cyclosporine (Sandimmune, Novartis-Pharma, Tokyo, Japan), 5 to 10 mg per kilogram of body weight, was begun one day before surgery and continued for at least one month postoperatively and thereafter if kidney function was normal.⁵ When trough levels of cyclosporine exceeded 150 ng per milliliter (125 nmol per liter), or when kidney function, liver function, or both were abnormal (serum creatinine, >1.5 mg per deciliter [133 μmol per liter]; blood urea nitrogen,



A



B



C

Figure 2. Results of Stem-Cell Transplantation in a 32-Year-Old Woman with the Stevens–Johnson Syndrome.

Panel A shows the preoperative conjunctivalized ocular surface, characterized by extensive vascularization. Panel B shows the intraoperative suturing of donor limbal tissue over the ocular surface. Panel C shows the condition of the ocular surface 17 months after surgery (the condition of the eye had not changed at the patient's latest visit, 20 months after surgery).

>30 mg per deciliter [10.7 μ mol per liter of urea]; or serum aspartate aminotransferase or alanine aminotransferase, >40 IU per deciliter), we reduced the dose of cyclosporine to less than 5 mg per kilogram or discontinued cyclosporine after one month. For patients who tolerated cyclosporine well, the trough levels were maintained between 30 and 100 ng per milliliter (24.9 and 83.1 nmol per liter) for more than six months. Cyclosporine diluted in α -cyclodextrin was also applied topically five times a day postoperatively, and the topical treatment was continued indefinitely unless toxic effects on the epithelium were suspected. Intravenous dexamethasone (Rinderon, Shionogi Pharmaceuticals, Tokyo, Japan), 8 mg per day, was given for the first 4 days after surgery; the dose was then tapered over the next 12 to 14 days. The topical corticosteroids used included betamethasone (Sanbetazone, Santen Pharmaceuticals, Osaka, Japan), applied five times a day for the first three months. The dose was then tapered to twice a day over the next three months.

Because the patients with ocular pemphigoid and the Stevens–Johnson syndrome had severe dry eye, proper wound healing could not be expected without tear supplementation. Eye drops prepared from autologous serum were used.¹⁴ Additional therapy for dry eye included the frequent use of preservative-free artificial tears, the wearing of eyeglasses with small, moistened sponges attached to special side panels,¹⁵ and the use of high-viscosity hyaluronate sodium eye drops (Hyalein, Santen Pharmaceuticals) five times a day. All patients' puncta lacrimale were occluded to increase tear retention on the ocular surface.

Evaluation of Efficacy

Surgical efficacy was evaluated on the basis of the success of corneal epithelialization and improvement in visual acuity. The former was based on three criteria: a clear appearance without epithelial defect on slit-lamp examination, the absence of abnormally high fluorescein permeability, and the absence of conjunctiva-derived goblet cells on impression cytology (a superficial cell biopsy). We considered the fulfillment of all three criteria to be an indication that the epithelium was of corneal origin. Each slit-lamp examination was carried out by the surgeon who had performed the operation, and the analyses of impression cytology, fluorescein permeability, and visual acuity were performed by personnel who were not aware of the surgical procedures used. Visual acuity was considered improved when at least two additional lines on the eye chart could be read (change in visual acuity, 0.1 to 0.3 or perception of hand motion to 0.01).

Evaluation of Safety

In assessments of postoperative complications, patients were examined for persistent epithelial defects, graft rejection, and ocular hypertension. Epithelial defects were considered persistent if they lasted more than two weeks. Most such defects were treated successfully by tarsorrhaphy (suturing of the eyelids), frequent use of autologous-serum eye drops, additional occlusion of the puncta lacrimale (occlusion of the efferent route of tear drainage to increase tear retention), or transplantation of amniotic membrane. Cases refractory to these treatments were considered incurable. Rejection of a corneal graft was diagnosed on the basis of a classic endothelial or epithelial appearance of rejection, accompanied by stromal edema in the transplanted corneal button. Because rejection restricted to stem cells alone is difficult to identify, rejection was considered a possibility only in cases in which combined lamellar (superficial) or penetrating (full-thickness) keratoplasty had been performed. Theoretically, stem-cell rejection is manifested as a persistent epithelial defect or eventual conjunctivalization; we did not include these conditions in our criteria for rejection, however. Ocular hypertension was considered a postoperative complication only when it had not been present preoperatively.

The primary measures of efficacy were the rate of successful corneal epithelialization at the most recent follow-up examination and improvement in visual acuity. Because transient amplifying cells (cells committed to differentiation to epithelium) in the corne-

al epithelium are believed to survive for only three to six months,^{16,17} the presence of corneal epithelialization beyond one year can be attributed to successful stem-cell transplantation.

Statistical Analysis

Best corrected visual acuity in the affected eye was measured at each clinical visit. For purposes of statistical analysis, counting fingers was categorized as an acuity of 0.004, hand motion as 0.002, light perception as 0.001, and no light perception as 0.0005. Final postoperative visual acuity was defined as the visual acuity at the most recent visit; the values were compared with preoperative values with use of the nonparametric Wilcoxon signed-rank test.¹⁸

RESULTS

We performed 73 stem-cell transplantations on 42 patients from March 1992 through July 1996. All operations were performed in the Department of Ophthalmology, Tokyo Dental College, Ichikawa General Hospital, Chiba, Japan. Three patients did not receive follow-up examinations for more than one year because they lived far away. Thus, we evaluated 70 procedures on 43 eyes from 39 patients (26 men and 13 women; mean age, 49 ± 23 years).

The average preoperative visual acuity was limited to "counting fingers," which satisfies the criteria for legal blindness in most countries. Only two patients had visual acuity better than 0.04: one patient with chemical injuries (0.2) and one patient with thermal injury (0.04). The average follow-up period was 3 years 68 days (1163 days; range, 365 to 2284 days).

We performed stem-cell transplantation as many as four times in a given eye if the cornea did not epithelialize or if there was a persistent epithelial defect. Table 1 shows the number of surgical procedures performed according to the cause of the dysfunction. For 24 of the 43 eyes, we performed only one operation. The incidence of repeated surgery was higher among the patients with chemical or thermal injury; 12 of 14 patients in this group had two or more procedures.

Efficacy

Twenty-two of the 43 eyes (51 percent) had corneal epithelialization after transplantation (Table 2). Of the 22 eyes, 7 had corneal stromal edema, and 15 (35 percent of the total number) had completely clear corneas (corneal epithelium with clear corneal stroma). Of the patients with the Stevens–Johnson syndrome or ocular pemphigoid, 41 percent had corneal epithelialization. Seventy-one percent of the patients with chemical or thermal injury had corneal epithelialization. Twenty-eight percent of the patients with the Stevens–Johnson syndrome or ocular pemphigoid and 50 percent of the patients with chemical or thermal injury had clear corneas.

Visual acuity improved by two or more lines on the acuity chart in 26 eyes (60 percent) (Fig. 3). Two patients with ocular pemphigoid or the Stevens–Johnson syndrome and two patients with chemical or thermal injury had a loss in visual acuity of two or more lines at the time of final examination. All four patients with decreased vision had corneal stromal edema; one eye had a persistent epithelial defect and another dense conjunctival epithelium. Mean visual acuity improved from counting fingers (0.004) to vision sufficient to distinguish the largest symbol on the visual-acuity chart from a distance of 1 m (0.02, $P < 0.001$). The 15 eyes in which the corneas became clear had a final mean visual acuity of 0.11 (the ability to distinguish the largest eye-chart symbol from a distance of 5 m).

Safety

Persistent epithelial defects (defects in the epithelium that did not resolve for more than two weeks) occurred in 26 eyes (60 percent) (Table 3); however, all but 2 eyes (5 percent), both in patients with the Stevens–Johnson syndrome or ocular pemphigoid, eventually healed (Table 2). When corneal transplan-

TABLE 2. OUTCOME OF CORNEAL EPITHELIAL STEM-CELL TRANSPLANTATIONS IN THE 43 EYES OF 39 PATIENTS, ACCORDING TO THE CAUSE OF DYSFUNCTION.

CHARACTERISTIC	STEVENS–JOHNSON SYNDROME OR OCULAR PEMPHIGOID	CHEMICAL OR THERMAL INJURY	TOTAL
No. of eyes	29	14	43
Corneal epithelialization — no. (%)	12 (41)	10 (71)	22 (51)
Conjunctival epithelium — no.	8	4	12
Skin-like epithelium — no.	7	0	7
Clear cornea — no. (%)	8 (28)	7 (50)	15 (35)
Untreatable persistent epithelial defect — no.	2	0	2
Final visual acuity			
Mean	0.02	0.04	0.02
Range	Light perception to 0.7	Light perception to 0.9	Light perception to 0.9

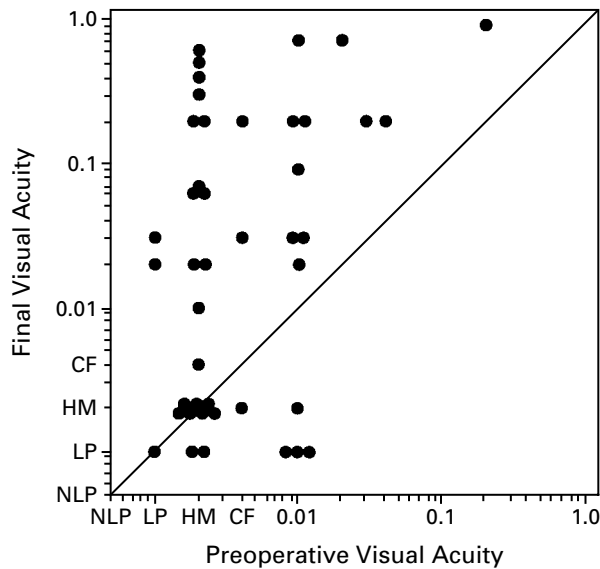


Figure 3. Changes in Visual Acuity after Surgery. NLP denotes no light perception, LP light perception, HM hand motion, and CF counting fingers. The diagonal line indicates the values at which the preoperative and postoperative values for visual acuity were the same.

tation was performed simultaneously with limbal transplantation, rejection of the corneal graft was also a major complication. The rate of corneal rejection was 69 percent among the patients with chemical or thermal injury and 27 percent among the patients with the Stevens–Johnson syndrome or ocular pemphigoid. Rejection was treated primarily medically, with intravenous and topical corticosteroids.

Regrafting of the cornea was performed as a final resort. Nine of the 13 eyes in which rejection occurred underwent a second transplantation. At the time of the patients’ latest visit, one of the nine re-grafted eyes had a clear cornea, one eye had corneal edema, and seven eyes had had a second episode of

rejection. Three of these seven eyes had clear corneas after medical treatment. Four eyes had received a third corneal transplant, which resulted in one clear cornea and three eyes with edema.

Ocular hypertension, which developed in 16 eyes (37 percent), was another common finding. Of the 16 eyes with ocular hypertension, 8 required surgery to reduce intraocular pressure; the other 8 were successfully treated with medication. Visual acuity decreased in 2 of the 16 patients and required surgical intervention.

DISCUSSION

We found that transplantation of corneal epithelial stem-cell grafts was efficacious for the treatment of severe ocular-surface disorders. The overall success rate — measured as the rate of corneal epithelialization — was 51 percent. Thirty-five percent of the corneas became completely clear. The patients with clear corneas had a final postoperative visual acuity of 0.11, which enabled them to perform daily activities. The overall average visual acuity of 0.02 provided patients with increased ocular function and greater self-sufficiency. Because the life span of transient amplifying cells (cells already present in the corneal epithelium and committed to epithelial differentiation) is believed to be less than one year — possibly less than three months^{16,17} — maintenance of a normal corneal epithelium for more than one year suggests sustained viability of the corneal stem-cell grafts. The longest follow-up period in the study was for a 56-year-old woman with ocular pemphigoid; her cornea has remained clear for more than four years. Epithelial cells originating in limbal grafts were identified by HLA class II typing (polymerase-chain-reaction analysis of restriction-fragment-length polymorphisms) in seven of nine patients included in the study who have been followed for more than one year. Only one of eight patients with corneal transplants had donor-derived cells after the same follow-up period.¹⁹

TABLE 3. POSTOPERATIVE COMPLICATIONS IN THE 43 EYES OF 39 PATIENTS, ACCORDING TO THE CAUSE OF DYSFUNCTION.*

COMPLICATION	STEVENS–JOHNSON	CHEMICAL OR	TOTAL
	SYNDROME OR	THERMAL INJURY	
	OCULAR PEMPHIGOID		
No. of eyes	29	14	43
Persistent epithelial defect — no. (%)	16 (55)	10 (71)	26 (60)
Ocular hypertension — no. (%)	8 (28)	8 (57)	16 (37)
Rejection of corneal graft — no./total no. (%)	4/15 (27)	9/13 (69)	13/28 (46)

*The complications are from the first corneal epithelial stem-cell transplantation in each eye, not from subsequent procedures.

The development of persistent epithelial defects in 60 percent of the corneas underlines the importance of postoperative care of the epithelium of the ocular surface. With proper treatment, the defects eventually healed in all but two corneas of patients with the Stevens–Johnson syndrome or ocular pemphigoid. The rejection of stem cells in the epithelium is difficult to detect, but a persistent epithelial defect may reflect such rejection. The overall rate of corneal-graft rejection in eyes into which corneas and stem cells were transplanted simultaneously was 46 percent.

Perhaps the most serious complication after surgery was ocular hypertension, which developed in 16 eyes (37 percent). The hypertension may have been related to the simultaneous removal of draining vessels, which are responsible for the outflow of aqueous humor from the eye, along with fibrous tissue. Both of the patients with decreased visual acuity after surgery to reduce intraocular pressure had opaque corneas as a result of edema on their final visit. Therefore, it was difficult to determine whether decreased vision was due to glaucoma or to the surgery.

In conclusion, we found that corneal epithelial stem-cell transplantation permits sustained reconstruction of the corneal epithelium in many eyes with severe primary disorders of the ocular surface. The control of persistent epithelial defects, ocular hypertension, dry eye, and graft rejection may further increase the efficacy of this method of transplantation.

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