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Auto Iris Pigment Epithelial Cell Transplantation in Patients with Age-Related Macular Degeneration: Short-Term Results

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Abe, T., Yoshida, M., Tomita, H., Kano, T., Sato, M., Wada, Y., Fuse, N., Yamada, T. and Tamai, M. *Auto Iris Pigment Epithelial Cell Transplantation in Patients with Age-Related Macular Degeneration: Short-Term Results.* Tohoku J. Exp. Med., 2000, **191** (1), 7-20 —— Autologous iris pigment epithelial cell transplantation was performed on patients with exudative age-related macular degeneration (AMD). Autologous IPE cell culture was performed using autologous serum after iridectomy in 7 patients with AMD. The cell suspensions ($2\sim20\times10^4$ cells) were transplanted into the submacular lesion of individuals after removal of neovascular membranes. Subsequent ophthalmological examinations, including best corrected visual acuity and fluorescein or indocyanine green angiography, were performed. In addition, 15 patients with AMD, who underwent removal of neovascular membrane without transplantation, were evaluated as non randomized controls. Varying degrees of atrophy or defects of choriocapillaris and retinal pigment epithelium were observed in all of the patients. No cystoid macular edema or fluorescein leakage was observed after treatment, but window defects were present. No patient had decreased visual acuity. One treated patient developed mild subretinal fibrosis and an other patient developed mild preretinal fibrosis, however no difference was significant when compared with the control. In coclusion, the treatment resulted in no significant improvement in macular function, as compared with the control; however, no rejection or deterioration in visual acuity occurred up to the 13 month follow up. —— age-related macular degeneration; transplantation; auto iris pigment epithelium; rejection

Transplantation of neural retina (Jiang et al. 1993), retinal pigment epithelium (RPE) (Lopez et al. 1989; Li and Turner 1991; Sheedlo et al. 1991; LaVail et al. 1992), and iris pigment epithelium (IPE) (Tamai 1996; Rezai et al. 1997a; Thumann et al. 1997) has been performed at the subretinal site using animal models such as Royal College of Surgeons (RCS) rats, whose original condition

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contributed to dysfunction of phagocytosis of the RPE (Bok and Hall 1971). Recently, the transplantation of fetal RPE cells has also been evaluated in human age-related macular degeneration (AMD) after the removal of subretinal neovascularization without immunosuppression. Some of the findings report a thickening of the macula, fluorescein leakage, and gradual decrease of visual acuity that may have been caused by graft rejection (Algvere et al. 1997). Peyman and coworkers also reported surgical excision of subfoveal neovascularization and scar tissue following the transplantation of autologous or homologous RPE with Bruch's membranes (Peyman et al. 1991). Autologous transplantation improved visual acuity, but homologous transplantation did not. Patients with homologous transplantation also showed encapsulation of the transplanted tissue. Zhang and Bok (1998) reported that systemic immunity, which may be derived through breakdown of blood-retinal barrier, appeared to exert a significant influence in the subretinal space. These results indicated that subretinal space is not always an immunologically privileged site (Niederkorn and Streilein 1986; Streilein 1990) and cells other than autologous cells may be rejected.

IPE has the same embryonic origin as RPE (McDonnel 1989) and may perform an alternative function similar to that of RPE. Rezai and coworkers (Rezai et al. 1997b) reported that IPE cells had a function of specific phagocytic capacity for the rod outer segment. IPE cells may also be able to form tight junctions in the subretinal space, thereby substituting for the blood-retinal barrier normally formed by RPE cells (Rezai et al. 1997c). Moreover, IPE transplantation delayed photoreceptor degeneration in RCS rats (Rezai et al. 1997a; Tamai et al. 1997).

The surgical removal of neovascular fibrous tissues in patients with AMD usually generates the removal of some or much of the local RPE layer (Lopez et al. 1991; Bynoe et al. 1994). To provide an alternate substance to function for defective RPE and to minimize host-graft rejection, we planned autologous cell transplantation using IPE, because IPE is easy to obtain from individuals. In this investigation, we studied the results of autologous IPE cell transplantation in AMD patients with submacular neovascular membranes.

Materials and Methods

Patients

Seven patients (2 females and 5 males) ranging in age from 49 to 85 years (average age, 69.6 years) with AMD participated in the study (Table 1). These patients were examined from September 1998 to May 1999, and informed consent was obtained from the all. All the patients had a unilateral lesion of AMD. The amount of time between the initial eye symptoms and transplantation ranged from 3 to 17 months (average 10). Preoperative visual acuity was between counting finger and 0.05. All of these patients had neovascular membranes that were detected either by fluorescein angiography (FAG) and/or indocyanine green

TABLE 1. *The characteristics of study patients*

	Age/ Sex	Duration (M)	Visual Acuity (pre/post)	NV (FAG)	NV (ICG)	Cells (culture days)	Cat (IOL)
1.	66/M	10 M	0.02/0.04	1.8×1.8	1.5×1.5	5×10^4 (39)	+
2.	49/F	12 M	0.05/0.1	0.6×0.6	0.5×0.5	10×10^4 (40)	−
3.	58/F	8 M	CF/0.04	1×1	1.5×1.5	2×10^4 (27)	−
4.	85/M	3 M	0.01/0.1	2×1	1×1	21×10^4 (35)	+
5.	70/M	5 M	0.02/0.04	0×0	0.2×0.2	10×10^4 (30)	−
6.	73/M	15 M	0.04/0.1	1×1	0.5×0.9	3×10^4 (40)	+
7.	77/M	17 M	0.04/0.04	0.3×0.5	1.5×1.5	5×10^4 (35)	−

The characteristics of study patients. CF indicates counting finger. Duration (M) indicates time (month) between onset of eye symptoms and subsequent transplantation. NV stands for neovascularization and the size was shown as disc diameter and cells and culture days mean cells used for transplantation and the number of days required for cell culture. Cat (IOL) indicates cataract surgery with intraocular lens implantation.

angiography (ICG). The size of the neovascular membrane was estimated to be between 0 (not detected in case 5) and 3 disc diameters (DD) by FAG and 0.2 and 2 DD by ICG. Follow up periods were between 6 and 13 months (average, 10.4). All of the patients were followed up without immunosuppression. For control, the visual acuity of 15 patients with AMD who had neovascular membranes

TABLE 2. *The characteristics of control patients*

	Age/ Sex	Duration (M)	Visual Acuity (pre/post)	NV		Cat (IOL)
				(FAG)	(ICG)	
1.	77/M	4	0.06/0.03	3×2	N.D.	+
2.	64/M	18	0.02/0.3	1×1	N.D.	+
3.	75/F	18	HM/HM	1×1	N.D.	+
4.	66/M	9	0.02/0.15	1×1	0.5×0.5	+
5.	46/M	30	0.03/0.01	1×1	0.5×0.5	−
6.	67/F	4	0.05×0.06	0.7×0.7	0.7×0.7	−
7.	77/M	60	0.02/0.01	0×0	3×3	+
8.	69/M	24	0.02/0.1	0.5×0.5	0.5×0.5	−
9.	65/M	30	0.08/0.15	1×1	0.3×0.3	−
10.	67/F	2	HM/0.05	0×0	3×3	−
11.	68/M	5	HM/0.1	1×1	1×1	−
12.	78/M	4	CF/0.07	N.D.	N.D.	−
13.	83/M	2	CF/0.1	0.5×0.4	0.5×0.5	+
14.	78/M	14	0.04/0.01	0.4×0.4	0.7×0.7	−
15.	65/M	2	HM/0.2	N.D.	N.D.	+

The characteristics of control patients. HM, hand motion; ND, examination was not performed. All other abbreviations are same as Table 1.

removed without transplantation was evaluated (Table 2). These patients were examined from November 1992 to September 1998 and were used retrospectively by non randomized comparative study. The duration between initial eye symptoms and surgery ranged from 2 to 60 months (average 13.4). The preoperative visual acuity ranged from hand motion to 0.08. The postoperative visual acuity and funduscopic evaluations were done between 6 and 14 months. The methods of operation are described here, and nothing differed between patients group except for cell transplantation. These studies were performed at Tohoku University Hospital.

Human research

The tenets of the Declaration of Helsinki were followed, and informed consent was obtained from all subjects who participated in this study after they were given an explanation of the nature and possible consequences of the study as well as the materials used for the study. This study was also approved by the ethics committee of Tohoku University, on January 26, 1998.

Clinical evaluations

Patients were examined ophthalmologically at Tohoku University Hospital. The subsequent ophthalmic examinations included best corrected visual acuity, slit lamp biomicroscopy, visual field analysis, fundus examination, and photography, FAG and ICG by scanning laser ophthalmoscopy (SLO) (Rodenstock, Munich, Germany), optic coherence tomography (OCT) (Humphrey, Carl Zeiss, Germany) and electroretinography (ERG).

Microperimetry of the macular area was also performed by SLO, if possible. The best corrected visual acuities were evaluated by the same physician in a masked fashion.

IPE cell preparation

Irises from the patients about to undergo transplantation were obtained after local anesthesia was performed. Peripheral iridectomy (about 3×3 mm) was conducted at 11 and/or 1 o'clock from the same eye about to undergo transplantation. The iris tissue was placed in balanced salt solution (BSS: Gibco BRL, Bethesda, MD, USA) immediately after obtaining it. IPE cell cultures were performed, using a modification of a method previously reported for RPE (Abe et al. 1998). In brief, iris segments were incubated in Dulbecco's solution in 0.25% trypsin at 37°C for 20 minutes. After incubation, the IPE was mechanically detached from the stroma, and pipetting was performed using a glass pipette. By pipetting gently under visualization with a dissecting microscope, we could collect isolated IPE in Ham's F-12 Nutrient Mixture (F-12; Gibco) supplemented with 15% autologous serum. Venous blood was collected from the same patient who was undergoing peripheral iridectomy. The blood sample was then centrifuged at

3500×g for 15 minutes at room temperature (RT) under sterile conditions to obtain serum alone. Next, the IPE cells were plated at 37°C in 5% CO_2 in 15% autologous serum in F-12 medium. The medium was changed every three days.

About 50% confluence of primary IPE cells was suspended using trypsin-ethylenediaminetetraacetic acid (EDTA) solution (0.125% trypsin and 0.2 mM EDTA) (Gibco), and the cells were collected by centrifugation after stopping the reaction with serum. The cells were washed with BSS 3 times and suspended in 15–20 μl of BSS for 30 minutes before the transplantation. The cells were counted using a Burker-Turk hemocytometer. The number of cells injected into the subretinal space was approximately $2\sim20\times10^4$ cells. The duration of the culture was between 27 and 60 days (average 38.3 days). All of these procedures were performed in a special "semi-sterile room" at the Department of Ophthalmology, Tohoku University.

Surgical procedures

Seven neovascular membranes from 7 patients with AMD were removed through a microretinotomy created after weak diathermal cauterization of the retina during 3-port vitrectomy. A tiny retinal bleb was made by injecting BSS

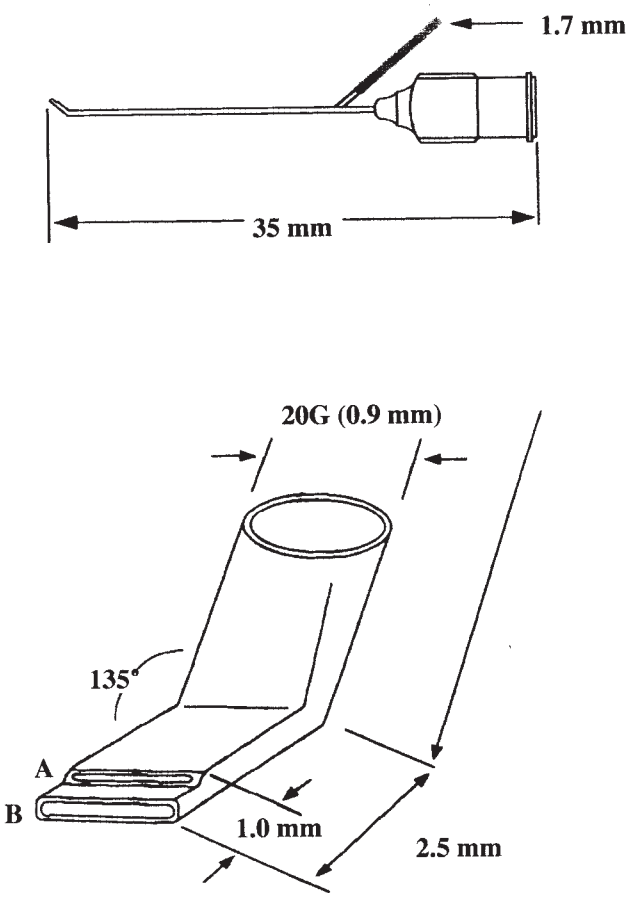


Fig. 1. An artist's drawing of the microinjection needle shows two orifices, one (A) for preinjection of BSS and aspiration, and the other (B) for injection of cultured cells.

through a small bent 30-gauge needle before extracting the neovascular membrane. The neovascular membrane was removed through the retinotomy. Next, the subretinal space was washed using a specially designed microinjection needle. The needle was made with two parts in one shaft, each of which could be controlled independently, one for BSS injection or aspiration (Tip A) and the other for cell injection (Tip B) (Fig. 1). Prior to the operation, those parts were filled with BSS and cultured IPE cells. Under microscopic control, the needle was inserted into the subretinal space by the surgeon after fluid-air exchange was performed. The assistant controlled the subretinal space by injecting or aspirating BSS into the subretinal space using Tip A and placed Tip B in the proper location and injected the cultured IPE cells in total 20 μl of BSS into the submacular area under microscopic control. All of the procedures were performed through microretinal holes, and the injection was performed by cell suspension. The microretinal holes were so small that photocoagulation was not needed. Three of the seven patients underwent lensectomy with an intraocular lens (IOL) implantation. After the procedure, the vitreous gel was exchanged with 20% of sulfur hexafluoride. The patients lay on their back for four hours and were positioned face down overnight. These procedures were performed by one surgeon. The control patients also underwent surgical removal of subretinal neovascularization without transplantation by two additional surgeons.

Immunohistochemistry

Cytokeratin immunocytochemistry was performed, as previously described (Abe et al. 1998). Briefly, preincubation was made with 0.3% hydrogen peroxide in a phosphate-buffered saline (PBS) solution for 20 minutes at RT. Incubation with the anti-cytokeratin was performed overnight at 4°C. Then the slides were incubated with the fluorescein-labeled second antibodies (goat anti-mouse IgG antibody) (Sigma, St. Louis, MO, USA) in Tween-PBS-2% fetal bovine serum for 30 minutes at RT. Following incubation with the second antibodies, the reactions were photographed with an OLYMPUS IMT-2 (Olympus, Tokyo). For a control, mouse IgG instead of the first antibodies was added to the incubation mixture containing Tween-PBS-2% FBS.

Statistical analysis

Statistical significance was determined by using the Mann-Whitney's U-test and Student's t-tests. A p less than 0.05 was considered significant.

Results

IPE cell cultures were successfully established from all of the patients (Figs. 2a and 2b show fresh and primary cultured IPE, respectively, from one patient). We confirmed that these cells were epithelial in origin by cytokeratin immunocytochemistry (Fig. 2c and 2d indicate test and control, respectively).

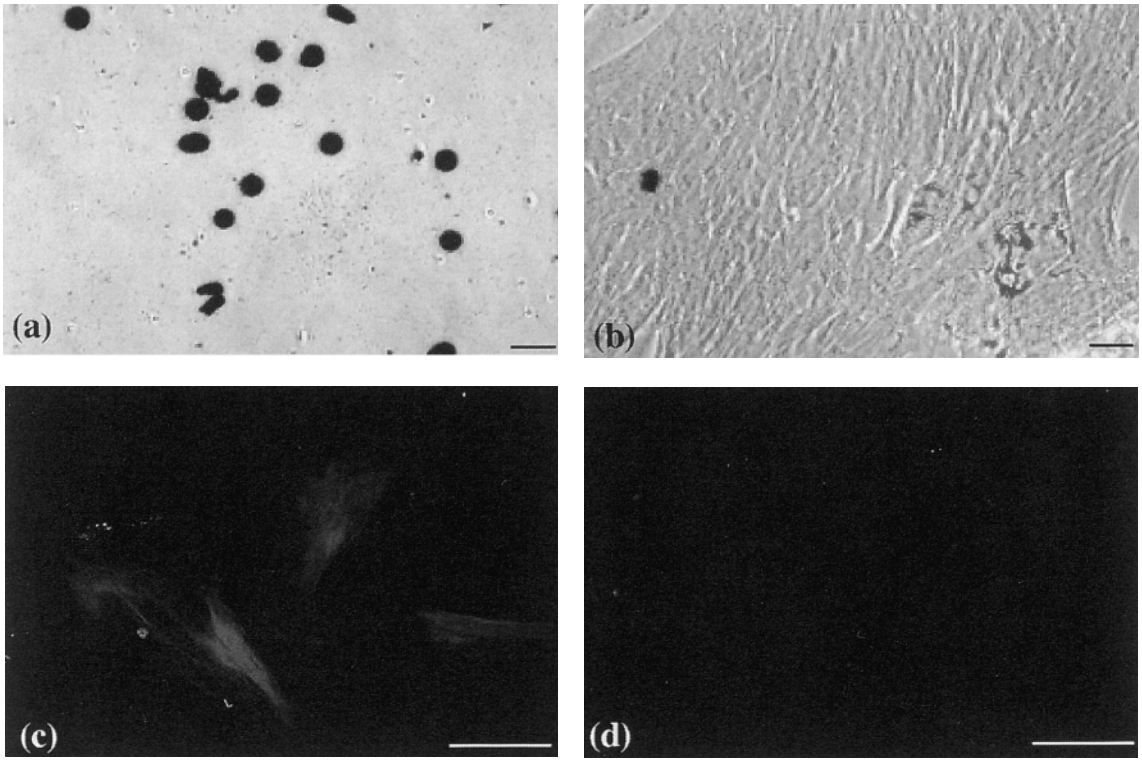
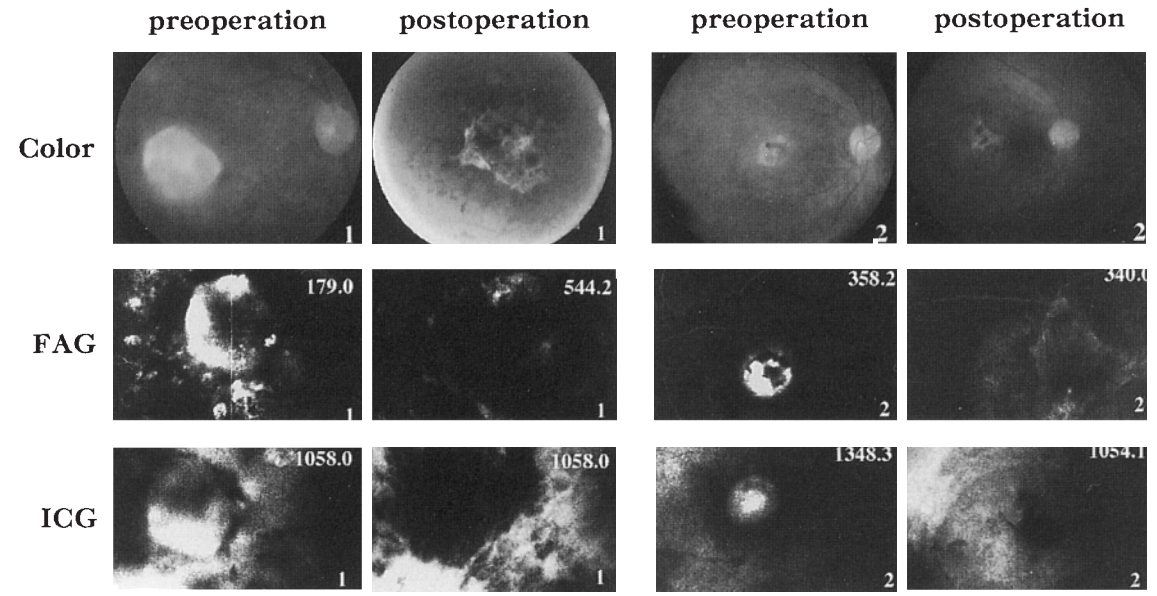


Fig. 2. IPE cell culture of one patient (Case 3) who underwent auto IPE cell transplantation. (a) IPE cells just prepared from the iris were obtained by peripheral iridectomy. (b) Primary culture from the same patient (27 days after culture was started). (c) Immunocytochemistry using cytokeratin antibodies of the same patient. (d) Immunocytochemistry without cytokeratin antibodies. Bar is 50 μm.

About 90% of the cells were stained by cytokeratin antibody. The cells used in the transplantation were $2\sim20\times10^4$ cells. The duration of the culture was between 27 to 60 days (average 38.3) (Table 1).

The duration between onset of eye symptoms and surgical treatment was 10 ± 5.1 months in patients, and 13.4 ± 15.6 in controls ($p=0.585$); the age was 68.3 ± 12.0 in patients and 69.7 ± 8.9 in controls ($p=0.765$); the preoperative visual acuity was 0.027 ± 0.017 in patients and 0.025 ± 0.023 in controls ($p=0.839$) (hand motion, and counting fingers were calculated as 0.004 and 0.006); the size of the neovascular membrane was 1.23 ± 0.66 in patients and 1.79 ± 1.71 in controls ($p=0.417$) and the cataract surgery with IOL implantation was 42.9% in patients and 46.7% in controls ($p=0.888$). No statistical significance was observed between the groups. All patients who participated in this study showed different amounts of submacular hemorrhage, exudate, fibrovascular membrane, and serous retinal detachment. Preoperative fundus photographs were taken of patients undergoing transplantation (Fig. 3). One patient (Case 1) received laser treatment outside of the macula area by another ophthalmologist for no known reason. In addition, all patients showed active neovascular membranes, which were confirmed either by FAG and/or ICG. Autologous IPE cell suspensions were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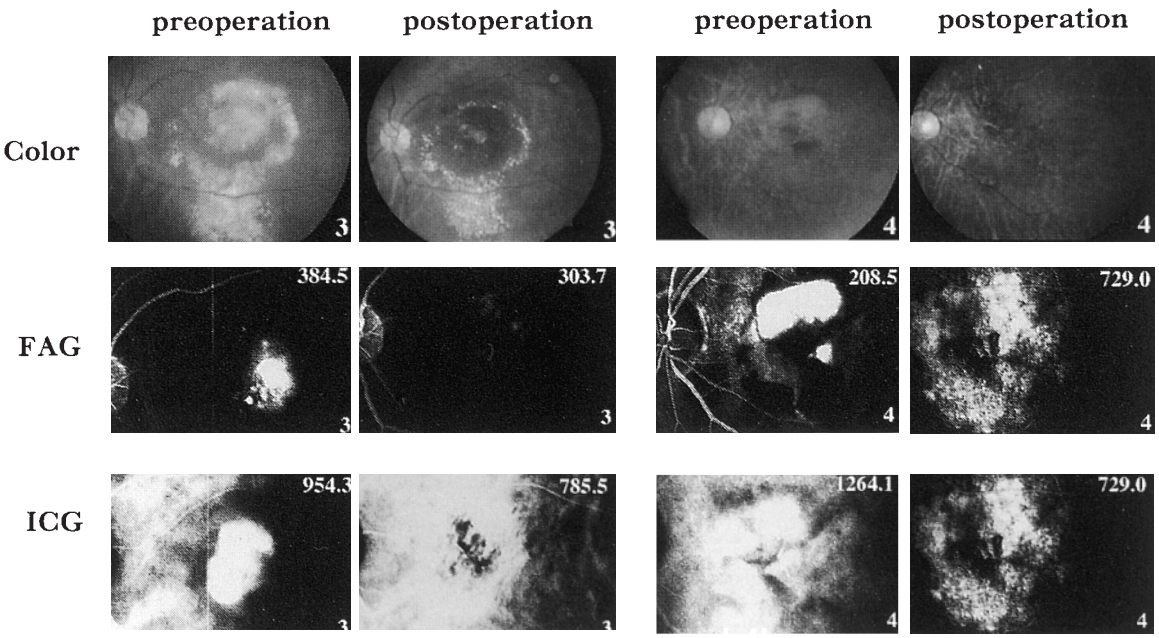
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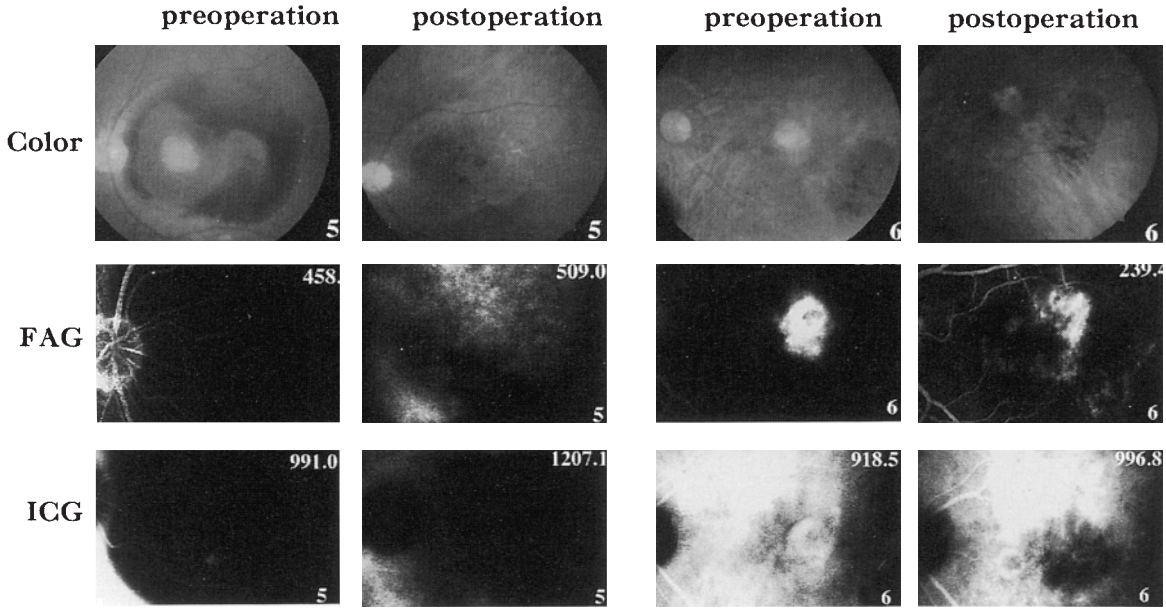

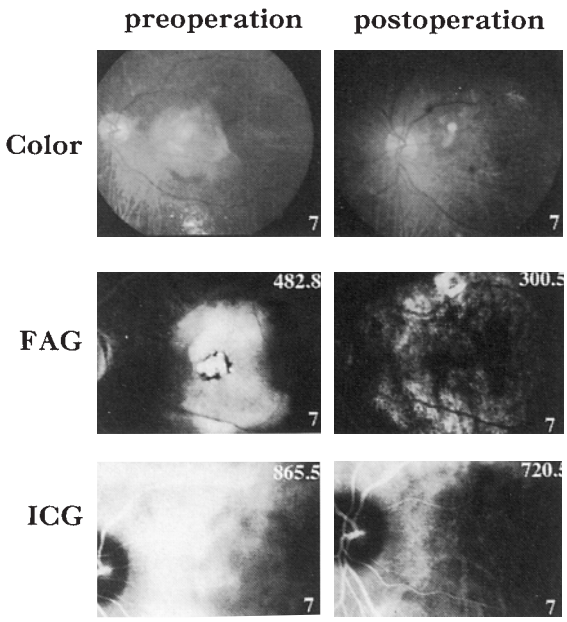

Fig. 3. (1)～(7) Preoperative and postoperative fundus photographs, FAG, and ICG of the patients who participated in this study are shown. Numbers appearing in the lower right corner correlate with the patient number in Table 1. Numbers in the upper right corner reflect the time after injection of either fluorescein or indocyanine green.

injected into the subretinal space after the removal of the neovascular membrane in these patients. We observed that the cells were injected into the submacular space by looking at the needle under a microscope. These patients were followed up without immunosuppression.

The photographs also show postoperative conditions between 6 and 13 months (average 10.4) after transplantation. No hemorrhage, exudate, or serous retinal detachment was observed. Also no neovascular membrane was observed either by funduscopic or FAG or ICG examination. However varying degrees of atrophy or defects of choriocapillaris and RPE were seen in these patients, which were detected by funduscopic or FAG or ICG examination. A faint hyperfluorescence (window defect) on FAG, probably due to injury or defect of RPE, was also observed in these patients. No distinct transplanted cells were visible by direct ophthalmoscopy or SLO or OCT examination. The results of microperimetry varied among the patients. One patient (Case 1) developed subretinal fibrosis (14%) and another patient (Case 3) developed mild preretinal fibrosis (14%), although results of a statistical analysis were not significant ($p = 0.823$).

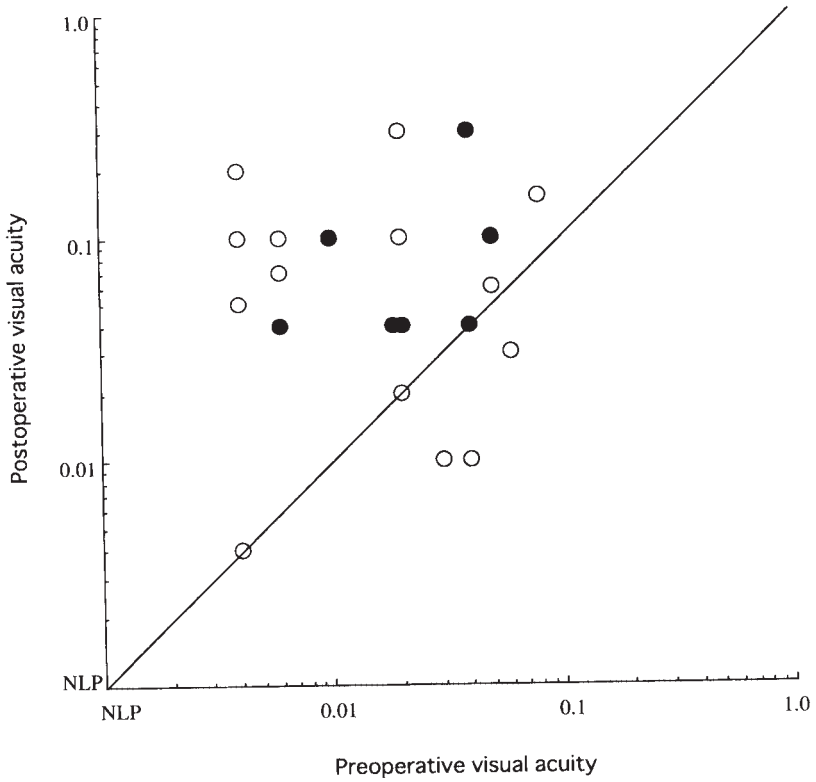

Fig. 4. Visual acuity of patients pretransplantation and posttransplantation. Closed circles indicate patients who underwent autologous IPE cell transplantation after removal of submacular neovascular membrane. Open circles portray control subjects who underwent only removal of submacular neovascular membrane without transplantation. The X and Y axes indicate pretransplantation and posttransplantation visual acuity, respectively. NLP indicates no light perception.

The average postoperative visual acuity was 0.094 ± 0.095 in the patients, and 0.116 ± 0.124 in the controls. A statistical analysis was not significant between the two groups ($p = 0.669$). Visual acuity pretransplantation and posttransplantation was plotted in Fig. 4 (closed circles). Control patients were also plotted in the same figure (open circle). Six of the seven patients showed improved visual acuity more than 2 lines (86%), and one patient maintained his preoperative visual acuity (14%). Conversely, approximately 13.3% showed decreased visual acuity, 73.4% improved, and 13.3% were the same in the control group. No patient in the transplantation group had decreased visual acuity, but statistical analysis was not significantly different between the two groups ($p = 0.438$).

The results of electrophysiological analysis, such as a-wave and b-wave amplitudes of a single flash ERG, photopic ERG, and flicker ERG were not statistically significant between preoperative and postoperative states (data not shown).

Discussion

Transplantation other than autologous cells may generate local rejection in patients with exudative AMD, even though the subretinal space has reportedly been an immunologically privileged site (Niederkorn and Streilein 1986; Streilein 1990; Zhang and Bok 1998). Consequent use of immunosuppression for preventing host-graft rejection may cause infection and toxicity (Wongpichedchai et al. 1992). Autologous RPE preparation from peripheral retina was reported in rabbit models (Wongpichedchai et al. 1992) or cadaver human eye (Ishida et al. 1998). However, it may be difficult to apply the technique in patients because it may require a difficult isolation technique and result in complications such as hemorrhage or infection. To minimize these problems, we performed autologous IPE cell transplantation in patients with AMD.

IPE is easy to obtain from the same individual and it grew well with autologous serum, although the proliferation rate of each cell was somewhat different among patients. IPE has the same embryonic origin and may also function alternatively as RPE. Recently, Schraermeyer and coworkers (Schraermeyer et al. 1999) reported that although transplanted fresh allologous IPE rescued photoreceptors significantly when compared to the untransplanted eye, the transplants were not as good as RPE transplants in short-term results. However, allologous IPE may influence the survival of the transplants (Zhang and Bok 1998). Kodama and Eguchi (1995) reported that transdifferentiation of the pigment epithelial cells in the eye are highly dependent on cell-cell or cell-extracellular matrix adhesion and communication. As Rezai and coworkers (Rezai et al. 1997b) speculated, transplantation of IPE cells in the subretinal space may create a new environment to transdifferentiate IPE cells into RPE cells. In this investigation, we used autologous cultured IPE cells for transplan-

tation.

To effectively transplant freshly prepared or cultured cells into subretinal space, the preparation and management of cells is important and occasionally critical for this process (Sheng et al. 1995; Seiler and Aramant 1998). In the case of cell transplantation in patients with AMD, vitreous surgery and extraction of subretinal neovascular membrane is at present not a difficult operation for vitreous surgeons. However, the method and instrument have develop during the successive periods and the procedure may influence the postoperative results. Our cases were from November 1992 to September 1998 for control and September 1998 to May 1999 for patients. The difference of the operative periods may influence the results. The operation was performed by three surgeons in our cases, which may also affect the results. These problems may be cleared by further prospective study. Also the proper transfer of these cells into subretinal space is critical. We have invented a unique needle to aspirate or inject fluid and to simultaneously inject cultured autologous IPE cells after removal of the neovascular membrane in patients with AMD. We could manage the place of cell injection properly. We could not, however, distinguish transplanted cells after transplantation, because they were in suspension and were less pigmented than the original IPE, eventhough we used SLO or OCT. Algvere and coworkers (Algvere et al. 1997) also reported that transplanted RPE cells by cell suspension were not visible, although they transplanted the cells in patients with nonexudative AMD.

Sheng and coworkers (Sheng et al. 1995) reported that fluorescein leakage is an early indicator of possibly showing host-graft rejection in animal transplantation experiments. In addition, Algvere and coworkers (Algvere et al. 1997) reported fluorescein leakage after transplantation in humans, implying host-graft rejection. No patients in our study showed fluorescein or indocyanine green leakage after transplantation. Although mild window defects were observed, as shown by other authors (Sheng et al. 1995; Algvere et al. 1997), making a microbleb at the subretinal space generates mild injury to RPE and creates a window defect observed on FAG. These window defects may be due to the injury or to a defect of RPE. We also observed no cystoid macular edema or gradually decreased visual acuity. These findings indicated no apparent host-graft rejection at the transplanted lesion in our patients. Although one patient showed mild preretinal fibrosis without fluorescein leakage and another patient showed mild subretinal proliferation, the frequency was not significant when compared to those of controls.

Because we performed cataract surgery with IOL implantation in four patients (42.8%), we tried to evaluate the results of transplantation not only by visual acuity, but by other examinations, such as visual field analysis and electrophysiological examinations. However, we could find no statistical significance between preoperation and postoperation, probably due to gross evaluation of macular function. As discussed here, these results may also reveal that

the transplant is not covering the area sufficiently and is not functioning as RPE in these short-term results.

Although autologous IPE transplantation was not significantly effective when comapred to control in short term follow up, no complication and rejection were observed. Maximum visual acuity of our patients was measured as 0.3. In the future, a careful selection of patients, such as preoperative visual acuity (less than 0.05 in our cases), the size of the neovascular membranes (maximum 3 DD in our cases), and duration of the disease (more than 3 months in our cases) may improve the results. Since these results derived from relatively early days of post transplantation, more time may be necessary to observe changes and function in the lesions.

Acknowledgments

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References

1) Abe, T., Sato, M. & Tamai, M. (1998) Dedifferentiation of the retinal pigment epithelium compared to the proliferative membranes of proliferative vitreoretinopathy. *Curr. Eye Res.*, **17**, 1103–1109.
2) Algvere, P.V., Berglin, L., Gouras, P., Sheng, Y. & Kopp, E.D. (1997) Transplantation of RPE in age-related macular degeneration: Observations in disciform lesions and dry RPE atrophy. *Graefes Arch. Clin. Exp. Ophthalmol.*, **235**, 149–158.
3) Bok, D. & Hall, M.O. (1971) The role of the pigment epithelium in the etiology of inherited retinal dystrophy in the rat. *J. Cell Biol.*, **49**, 664–682.
4) Bynoe, L.A., Chang, T.S., Funata, M., Del Priore, L.V., Kaplan, H.J. & Green, W.R. (1994) Histopathologic examination of vascular patterns in subfoveal neovascular membranes. *Ophthalmology*, **101**, 1112–1117.
5) Ishida, M., Lui, G.M., Yamani, A., Sugino, I.K. & Zarbin, M.A. (1998) Culture of human retinal pigment epithelial cells from peripheral scleral flap biopsies. *Curr. Eye Res.*, **17**, 392–402.
6) Jiang, L.Q., Jorquera, M. & Streilein, J.W. (1993) Subretinal space and vitreous cavity as immunologically privileged sites for retinal allografts. *Invest. Ophthalmol. Vis. Sci.*, **34**, 3347–3354.
7) Kodama, R. & Eguchi, G. (1995) From lens regeneration in the newt to in-vitro transdifferentiation of vertebrate pigmented epithelial cells. *Seminor Cell Biol.*, **6**, 143–149.
8) LaVail, M.M., Li, L., Turner, J.E. & Yasumura, D. (1992) Retinal pigment epithelial cell transplantation in RCS rats: Normal metabolism in rescued photoreceptors. *Exp. Eye Res.*, **55**, 555–562.
9) Li, L. & Turner, J.E. (1991) Optimal conditions for long-term photoreceptor cell rescue in RCS rats: The necessity for healthy RPE transplants. *Exp. Eye Res.*, **52**, 669–679.
10) Lopez, R., Gouras, P., Kjeldbye, H., Sullivan, B., Reppucci, V., Brittis, M., Wapner, F. & Goluboff, E. (1989) Transplanted retinal pigment epithelium modifies the

retinal degeneration in the RCS rat. *Invest. Ophthalmol. Vis. Sci.*, **30**, 586–588.
11) Lopez, P.F., Grossniklaus, H.E., Lambert, H.M., Aaberg, T.M., Capone, A., Jr., Sternberg, P., Jr. & L'Hernault, N. (1991) Pathologic features of surgically excised subretinal neovascular membranes in age-related macular degeneration. *Am. J. Ophthalmol.*, **112**, 647–656.
12) McDonnel, J.M. (1989) Ocular embryology and anatomy. In: *Retina*, edited by S.J. Ryan & T.E. Ogden, Vol. 1, St. Louis, CV Mosby, 5–15.
13) Niederkorn, J.Y. & Streilein, J.W. (1986) Lymphoma allografts abrogate immune privilege within the anterior chamber of the eye. *Invest. Ophthalmol. Vis. Sci.*, **27**, 1235–1243.
14) Peyman, G.A., Blinder, K.J., Paris, C.L., Alturki, W., Nelson, N.C., Jr. & Desai, U. (1991) A technique for retinal pigment epithelium transplantation for age-related macular degeneration secondary to extensive subfoveal scarring. *Ophthalmic Surg.*, **22**, 102–108.
15) Rezai, K.A., Kohen, L., Wiedemann, P. & Heimann, K. (1997a) Iris pigment epithelium transplantation. *Graefes Arch. Clin. Exp. Ophthalmol.*, **235**, 558–562.
16) Rezai, K.A., Lappas, A., Farrokh-siar, L., Kohen, L., Wiedemann, P. & Heimann, K. (1997b) Iris pigment epithelial cells of long evans rats demonstrate phagocytic activity. *Exp. Eye Res.*, **65**, 23–29.
17) Rezai, K.A., Lappas, A., Kohen, L., Wiedemann, P. & Heimann, K. (1997c) Comparison of tight junction permeability for albumin in iris pigment epithelium and retinal pigment epithelium in vitro. *Graefes Arch. Clin. Exp. Ophthalmol.*, **235**, 48–55.
18) Schraermeyer, U., Kociok, N. & Heimann, K. (1999) Rescue effects of IPE transplants in RCS rats: Short-term results. *Invest. Ophthalmol. Vis. Sci.*, **40**, 1545–1556.
19) Seiler, M.J. & Aramant, R.B. (1998) Intact sheets of fetal retina transplanted to restore damaged rat retinas. *Invest. Ophthalmol. Vis. Sci.*, **39**, 2121–2131.
20) Sheedlo, H.J., Li, L. & Turner, J.E. (1991) Photoreceptor cell rescue at early and late RPE-cell transplantation periods during retinal disease in RCS dystrophic rats. *J. Neural Trans. Plast.*, **2**, 55–63.
21) Sheng, Y., Gouras, P., Cao, H., Berglin, L., Kjeldbye, H., Lopez, R. & Rosskothen, H. (1995) Patch transplants of human fetal retinal pigment epithelium in rabbit and monkey retina. *Invest. Ophthalmol. Vis. Sci.*, **36**, 381–390.
22) Streilein, J.W. (1990) Anterior chamber associated immune deviation: The privilege of immunity in the eye. *Surv. Ophthalmol.*, **35**, 67–73.
23) Tamai, M. (1996) Retinal pigment epithelial cell transplantation: Perspective. *Acta Soc. Ophthamol. Jpn.*, **100**, 982–1006.
24) Tamai, M., Takeda, Y., Yamada, K., Tomita, H., Abe, T., Kojima, S. & Ishigura, S. (1997) bFGF transfected iris PE may rescue photoreceptor cell degeneration in RCS rat. In: *Retinal Degeneration*, edited by M.M. LaVail, R.E. Anderson & J.G. Hollyfield, Plenum Press, New York, 323–328.
25) Thumann, G., Schraermeyer, U., Bartz-Schmidt, K.U. & Heimann, K. (1997) Descemet's membrane as membranous support in RPE/IPE transplantation. *Curr. Eye Res.*, **16**, 1236–1238.
26) Wongpichedchai, S., Weiter, J.J., Weber, P. & Dorey, C.K. (1992) Comparison of external and internal approaches for transplantation of autologous retinal pigment epithelium. *Invest. Ophthalmol. Vis. Sci.*, **33**, 3341–3352.
27) Zhang, X. & Bok, D. (1998) Transplantation of retinal pigment epithelial cells and immune response in the subretinal space. *Invest. Ophthalmol. Vis. Sci.*, **39**, 1012–1027.