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CLINICAL INVESTIGATION

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Iris pigment epithelial cell translocation in exudative age-related macular degeneration

A pilot study in patients

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Abstract *Background:* This prospective, non-controlled pilot study investigates the practicability of IPE translocation and functional outcome in ARMD patients. Removal of submacular choroidal neovascularization (CNV) in age-related macular degeneration (ARMD) is usually associated with RPE damage and poor visual prognosis. Homologous RPE transplants fail to preserve macular function, possibly due to immune rejection. Instead of homologous RPE, we suggest translocating autologous iris pigment epithelium (IPE), building on earlier evidence from animal and in vitro investigations that IPE can substitute RPE functions in the experimental animal. Immunological cell rejection is avoided.
Methods: Four eyes with well-defined and eight eyes with ill-defined subfoveal CNV were submitted to operation and followed up for a minimum of 6 months. IPE cells were harvested from a peripheral iridectomy. A vitrectomy was performed. Submacular membranes were removed, and isolated IPE cells were injected into the subretinal space. Examinations included ETDRS visual acuity, fluorescein angiography, and SLO microperimetry.
Results: All patients underwent successful surgical removal of CNV and subretinal IPE injection. Compared to preoperative visual acuity (20/400–20/100) no significant change was observed after 6 months (20/320–16/80). A change of more than two ETDRS chart lines was defined as significant. One eye with preoperative ill-defined CNV developed a recurrence, leading to reduced visual acuity. In all patients, postoperative fluorescence angiography revealed early hyperfluorescence (window defect) in the surgically denuded area. Central fixation was demonstrated in 50% of eyes.
Conclusions: Preliminary data suggests that IPE translocation in submacular surgery for ARMD can preserve but not improve preoperative visual acuity over 6 months. Functional results are promising compared to submacular membrane extraction alone and RPE transplantation. Continued research on improvement of IPE translocation seems justified.

Introduction

In age-related macular degeneration (ARMD), malfunction of the RPE, and the Bruch's membrane–choriocapillariscomplex is followed by degeneration of macular photoreceptors, leading to loss of central visual acuity. In its exudative form ARMD is accelerated by choroidal neovascularization into the subretinal space. Surgical removal of subfoveal neovascular membranes invariably damages the RPE. Since photoreceptor function depends on an intact RPE cell layer, transplantation of RPE cells into the subretinal space has been suggested. While heterologous RPE cell transplantation was successful in monkeys, rabbits [7, 22] [12] and rats [18–20, 35], ho-

mologous fetal RPE transplantation in man failed after 1–3 months, presenting as cystoid macular edema [1, 2]. It was assumed that the transplants were immunologically rejected [1, 2, 11]. Prophylactic immunosuppression, however, is hardly tolerated in old age.

We investigated autologous IPE translocation as an alternative to homologous RPE transplantation to avoid immunological rejection and because the IPE is easily accessible by iridectomy. Several investigations suggest that IPE can take on RPE functions. The ability of IPE to phagocytose rod outer segments is 70% that of to RPE [29]. IPE can form tight junctions in vivo and in vitro [8–10, 30] and expresses molecules involved in retinol metabolism [3]. IPE cells that were transplanted into the subretinal space of RCS rats reduced the rate of degeneration of photoreceptor cells [28, 34]. The reduced rate of degeneration after transplantation of IPE cells into the subretinal space was significant when compared to sham-injected eyes (according to the technique of Silverman and Hughes [36]) after 90 days [28]. We therefore studied whether autologous IPE translocation is useful in conjunction with submacular membrane extraction in 12 patients with exudative ARMD.

Patients and methods

Patients

We performed IPE translocation in 12 patients with exudative ARMD (age 60–84, mean 73.1±7.51 years) with subfoveal CNV after informed consent. The subfoveal neovascular membranes were not amenable to laser photocoagulation according to the Macular Photocoagulation Study Group (MPS) [23–26]. Inclusion required angiographic evidence of subfoveal choroidal neovascularization, with ill-defined subfoveal complex or well-defined CNV larger than 2 MPS disc areas. The size of the CNV and the postoperative RPE defect was estimated using the MPS "disc area template" [23].

An ophthalmologic examination was performed pre- and postoperatively at several time points up to 6 months. The determination of best corrected visual acuity was based on the Early Treatment Diabetic Retinopathy Study (ETDRS) chart. Slit-lamp examination, color fundus photography and fluorescein angiography were performed prior to surgery and repeatedly during follow-up.

Macular microperimetry was performed using the Rodenstock scanning laser ophthalmoscope (SLO; Rodenstock, Germany) with refined Aachen software [40]. As visual stimuli, small single-fixation cross signs (10×10 to 30×30 pixels) were used. Each fixation test involved an adaptation of the retinal image to the presented fixation cross with a retinal landmark. Test stimuli were presented for 200 ms. An infrared laser (720 nm) was used to image the fundus on a video monitor. Background illumination was produced by a 633-nm-helium neon laser. The microperimetrically determined fixation site was transferred to the fundus photographs and angiograms to relate it to the estimated fovea and the surgical scar.

Methods

Two peripheral iridectomies were performed in the affected eye. Preparation of IPE cells followed a modification of the technique of Hu and co-workers [14, 15]: The small pieces of iris were unfolded in a drop of balanced salt solution (BSS) with the pigmented side facing upwards. The IPE cell layer was removed by gently scraping off a continuous sheet. The sheet was washed carefully in BSS solution to minimize contamination with cells of the iris stroma. The double cell layer was then cut into smaller pieces and suspended in 100 µl of BSS with 20% serum from the same patient. The suspension was transferred to an Eppendorf vial. Gentle shaking of the vial allowed dissociation of IPE into single IPE cells, several small cell clusters and some cell sheets. According to Hu and co-workers [15], our in vitro experiments showed that after mechanical isolation single cells and especially small cell clusters were able to form well-proliferating colonies.

Prior to surgery, we verified the viability and purity of isolated IPE cells. In vitro experiments had been performed with specimens of iridectomies to evaluate the cell number. A mean of 36000 cells (±17,920) were gained from each iridectomy. Viability testing was performed in a modification of the technique of Hu and co-workers [15] using the dye fluorescein diacetate [31]. Viability of 75.45% (±11.34%) was measured. The purity of the IPE cell suspension was tested by phase-contrast microscopy. More than 95% of all isolated cells were pigmented. Cell cultures made of isolated cell suspensions that were incubated with DMEM medium supplemented with 20% FCS at 37°C with 5% CO_2 showed that only pigmented cells and no fibrocytes had grown. This result was confirmed by immunocytochemistry with anticytokeratin antibodies (Sigma, monoclonal anti pancytokeratin, C 2931). The isolated pigmented cells showed positive fluorescence.

Pars plana vitrectomy with posterior vitreous separation was performed. A pointed, 20-µl-glass micropipette (Sigma), connected to a 2.0-ml syringe filled with BSS by a flexible tube, was used to penetrate the neurosensory retina about 3–3.5 mm temporal to the fovea and slightly superior to the horizontal raphe. The retina was perforated and detached by the jet stream of the pipette to form a retinal bleb. Through the same retinotomy, the CNV was mobilized and extracted by means of horizontally opening submacular microforceps (Dutch Ophthalmic Research CenterNetherlands). To seal the now somewhat enlarged retinotomy, a small bubble of liquid perfluorocarbon (perfluorodecalin) was placed over the retinotomy. The remaining subretinal fluid was thereby trapped and the wall of the retinal bleb tightened. The IPE cell suspension was aspirated into the micropipette. The cells were injected into the subretinal space through the peak of the retinal bleb with a small amount of reflux. Typically, a darkish-brown cloud became visible under the retina. Finally, the PFCL was removed and an air fluid–gas exchange was performed. Patients were postured supine on the day of surgery and prone for the next day.

Postoperative follow-up examination at 6 weeks, 3 months and 6 months included ETDRS visual acuity, slit-lamp biomicroscopy, tonometry, fundoscopy, SLO microperimetry and fluorescein angiography.

Results

Four of the 12 patients had a well-defined choroidal neovascularization (initial visual acuity 20/100–20/400) the others had an ill-defined neovascular complex (initial visual acuity 20/100–20/400), which was associated with a pigment epithelial detachment in four cases. Three of the 12 patients (nos. 1, 3, 7) had a history of radiation therapy in the treated eye.

The mean duration of visual loss prior to surgery was 6.6 months (±4.7 months). Visual acuity ranged from 20/100 to 20/400 preoperatively and from 16/80 to 20/320 postoperatively (Table 1). Significant change in

Table 1 Individual patient data (*CNV ill* ill-defined choroidal neovascularization, *CNV well* well-defined choroidal neovascularization, *PED* Pigment epithelial detachment)

Patient	Age (years)	Duration of visual loss (months)	Diagnosis	Visual acuity, pre-operative, ETDRS	Visual acuity, post-operative, 6 months ETDRS	Visual acuity, best ETDRS	Fixation, postoperative
1	80	12	PED	20/63	20/80	20/80	Extrafoveal
2	72	12	CNV ill	20/80	20/250	20/250	Foveal
3	72	6	CNV well	20/100	20/160	20/160	Extrafoveal
4	65	1	CNV ill	20/100	20/160	20/160	Foveal
5	60	2	PED	20/125	20/160	20/100	Foveal
6	66	4	PED	20/250	20/200	20/200	Foveal
7	84	12	CNV ill	20/250	20/250	20/250	Extrafoveal
8	73	10	CNV well	20/320	20/320	20/320	Foveal
9	78	2	CNV well	20/400	20/320	20/320	Extrafoveal
10	77	1	CNV well	20/400	20/250	20/250	Extrafoveal
11	83	5	PED	20/400	20/400	20/400	Foveal
12	68	12	CNV ill	20/400	20/320	20/200	Extrafoveal

visual acuity was defined as a change of at least two ETDRS lines. According to this classification, 11 eyes remained unchanged and one eye worsened. Eight of 12 patients reported a reduction in scotoma size and "brighter" vision. Central fixation (SLO microperimetry) was registered preoperatively in eight patients and maintained in six patients throughout the follow-up time of 6 months. Fixation was near pigmented deposits that did not exist preoperatively. There was no preferred location for extrafoveal fixation (six patients), which was documented at the superior, inferior, nasal or temporal margin of the surgical scar.

Metamorphopsia, when present, subsided in all but one patient, who developed recurrent neovascularization.

Bleeding from retinal vessels during membrane extraction occurred when the choroidal membrane was very adherent to the retina (patients 1, 2, 10, 11, 12). Three of those five patients reported a decrease in visual acuity over a period of more than 12 months duration (patients 1, 2, 12). When there was no bleeding, as in 7 cases, only one patient (no. 7) had a decrease in visual acuity over a period of more than 12 months duration.

Injection of IPE cell suspension was successful in every eye. Sometimes, however, when the retinal bleb was overfilled with fluid, a small amount of cells was washed back into the vitreous cavity. These cells were removed via a flute needle. Postoperatively, translocated IPE was often difficult to identify. In some cases dispersed pigmentation or pigment patches could be observed at the posterior pole, and sometimes additional pigmentation was found under the inferior temporal vascular arcade. It remained unchanged throughout the follow-up.

The typical fluorescein angiographic finding after membrane extraction was a hyperfluorescent area without leakage corresponding to the surgical RPE defect. The size of vascularized PED (pigment epithelial detachment) and well-defined CNV (as demonstrated by fluorescein angiography) corresponded to the postoperative RPE defect. Preoperatively, the mean size of CNV was 3.125±0.75 MPS disc areas for well-defined CNV and 4.4±2.68 MPS disc areas for PED. Postoperatively, the mean RPE defect was 3.25±0.87 MPS disc areas for well-defined CNV and 4.5±2.65 MPS disc areas for PED. The mean pre- to postoperative difference was 0.25±0.29 MPS disc areas in well-defined CNV and 0.1±0.22 MPS disc areas in PED. However, in ill-defined CNV the area of postoperative RPE defect was greater by 1.67±0.29 MPS disc areas than the preoperative size of the CNV.

One eye was complicated by a recurrent membrane (see report of case 4 below).

We did not observe macular edema or macular pucker formation. All eyes were phakic and developed a myopic shift in refraction (mean –3.4±.8 dptr) along with increased lens opacification. Most patients mentioned that they were dazzled by bright light, which is most likely due to increased cataract formation and large iridectomies.

Case reports

Case 1: patient 6

A 66-year-old woman presented with a PED and a neovascular membrane (Fig. 1a). In the previous 4 months, her visual acuity had dropped to 20/250 and she was complaining of metamorphopsia in the left eye. Fixation was foveal. Intraoperatively, IPE cells were harvested from a superior iridectomy. The subfoveal neovascular complex was removed from under a retinal bleb through a temporal paramacular retinotomy, and 0.1 ml of IPE cell suspension was injected, while the retinotomy site was blocked by 0.5 ml perfluorodecalin. The procedure was concluded by a fluid–air exchange.

The visual acuity was 20/200 6 weeks later and remained stable throughout the follow-up of 6 months. Microperimetry showed preserved foveal fixation (Fig. 1c). Pre-existing lens opacity pro-

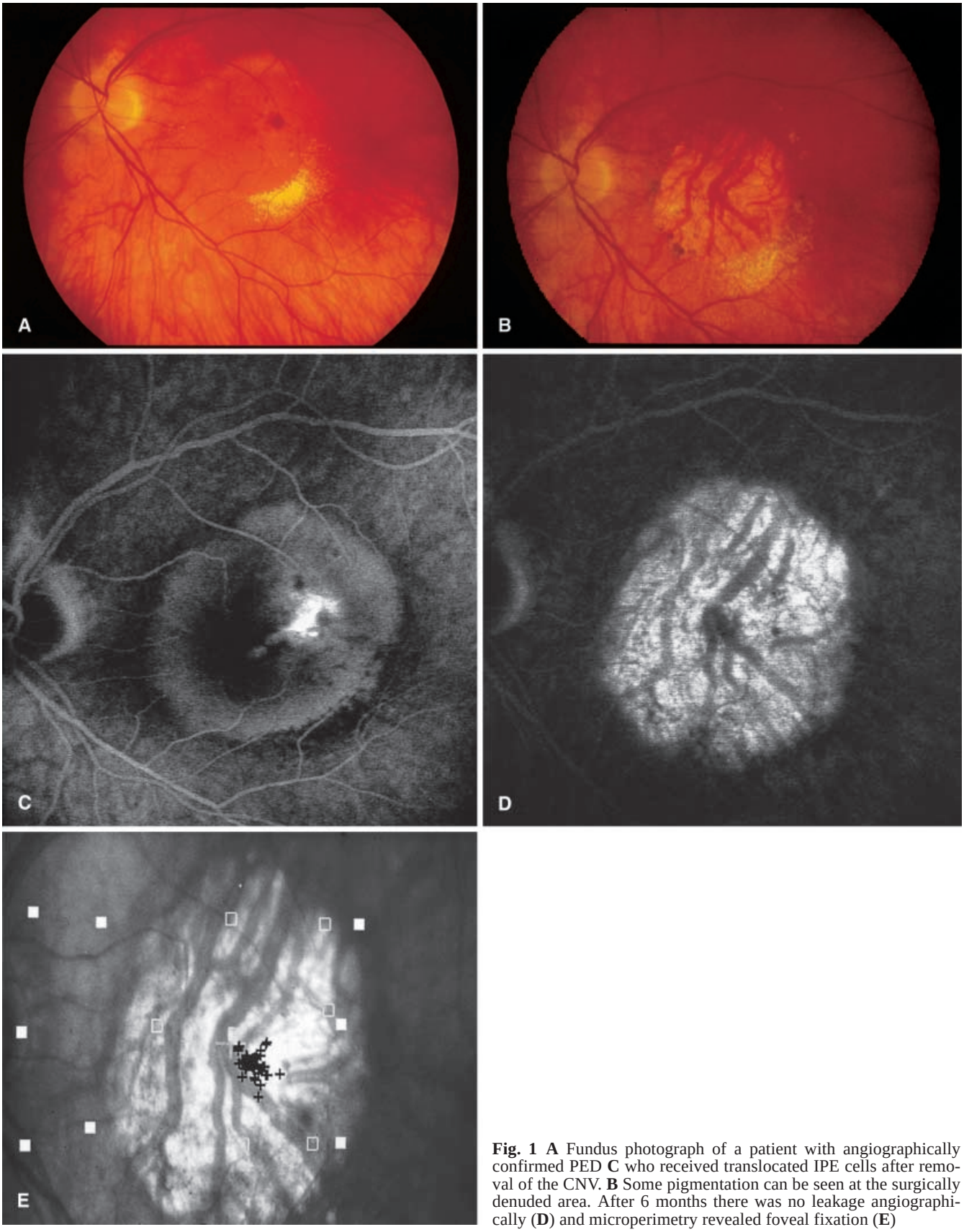

Fig. 1 **A** Fundus photograph of a patient with angiographically confirmed PED **C** who received translocated IPE cells after removal of the CNV. **B** Some pigmentation can be seen at the surgically denuded area. After 6 months there was no leakage angiographically (**D**) and microperimetry revealed foveal fixation (**E**)

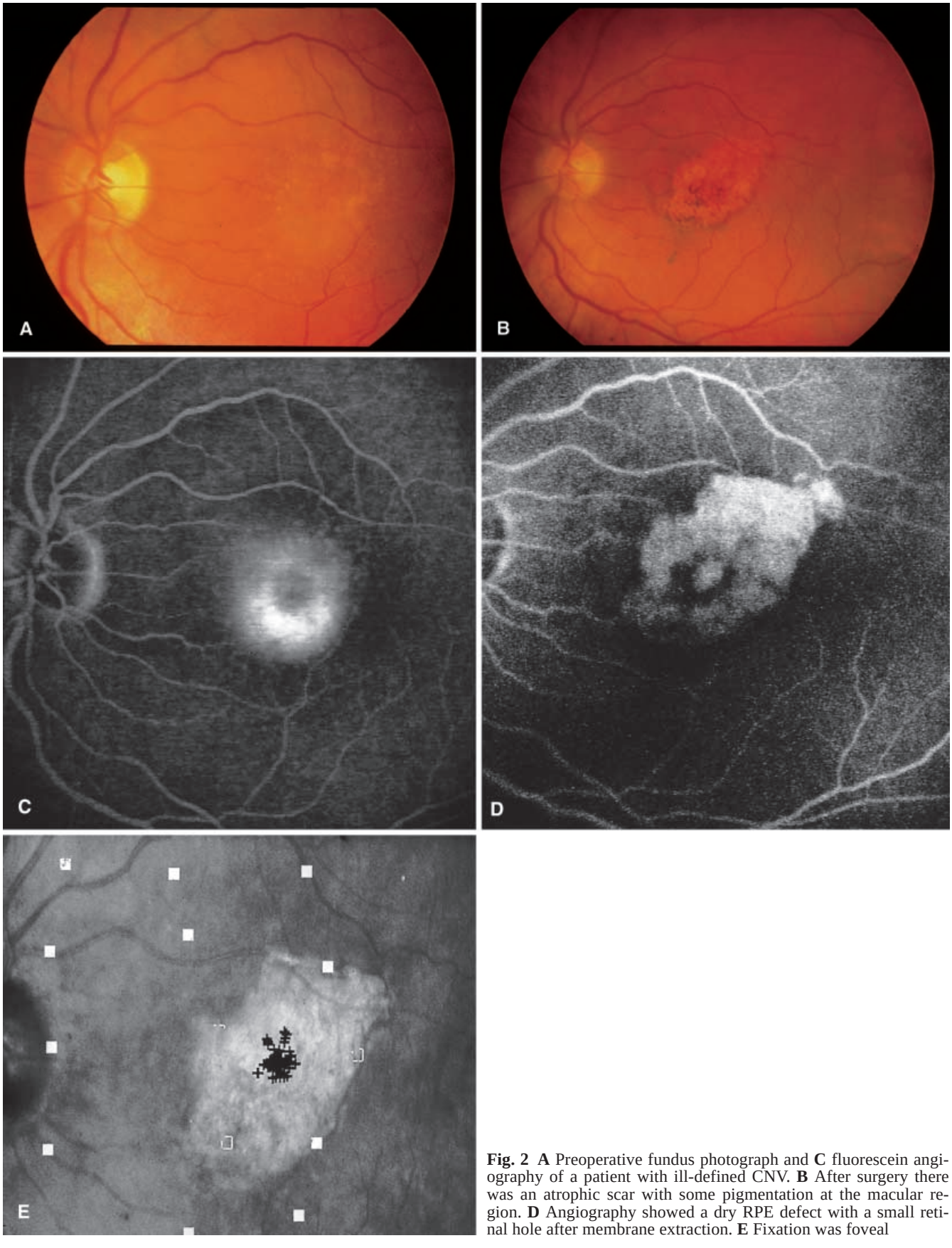

Fig. 2 **A** Preoperative fundus photograph and **C** fluorescein angiography of a patient with ill-defined CNV. **B** After surgery there was an atrophic scar with some pigmentation at the macular region. **D** Angiography showed a dry RPE defect with a small retinal hole after membrane extraction. **E** Fixation was foveal

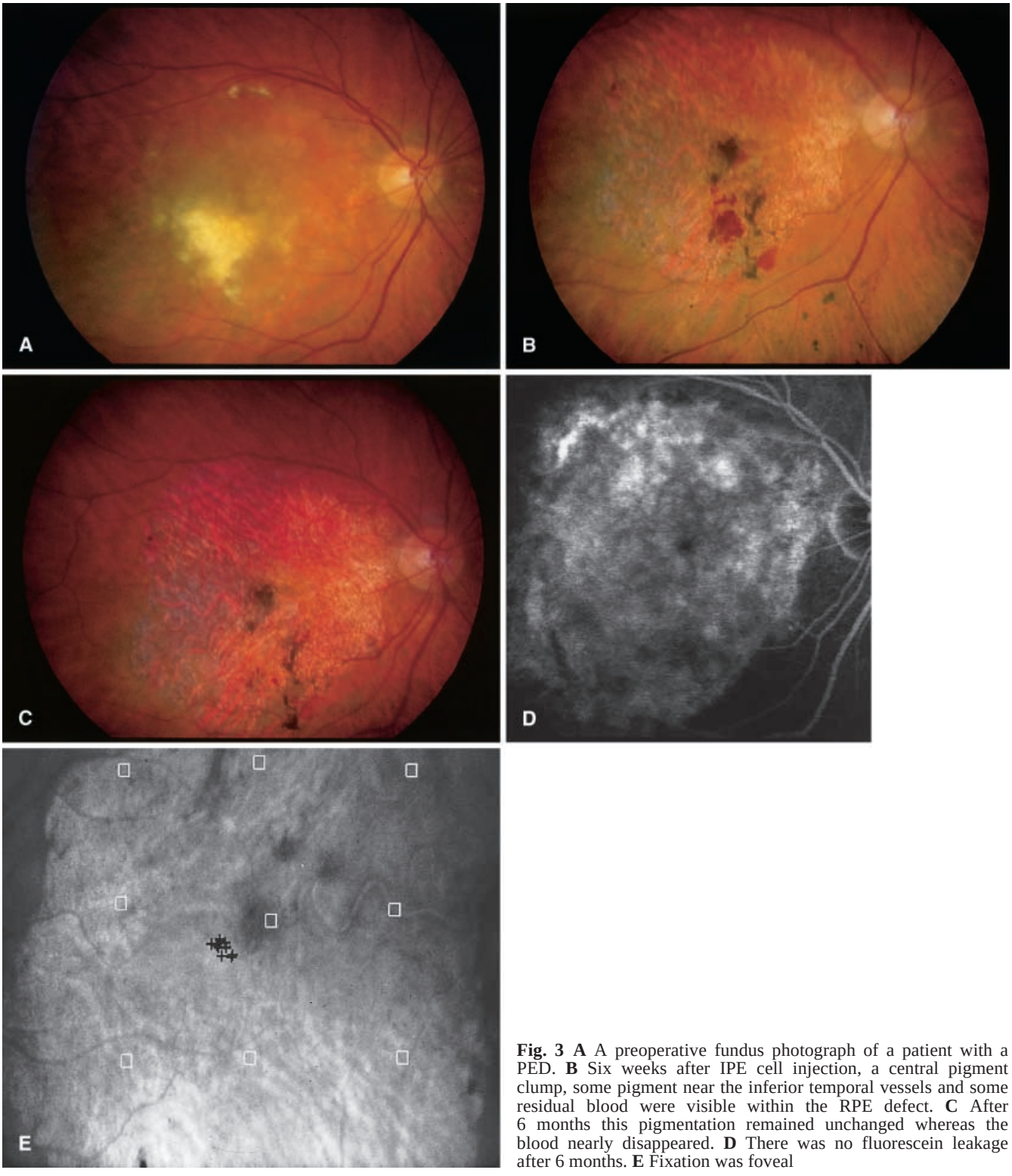

Fig. 3 **A** A preoperative fundus photograph of a patient with a PED. **B** Six weeks after IPE cell injection, a central pigment clump, some pigment near the inferior temporal vessels and some residual blood were visible within the RPE defect. **C** After 6 months this pigmentation remained unchanged whereas the blood nearly disappeared. **D** There was no fluorescein leakage after 6 months. **E** Fixation was foveal

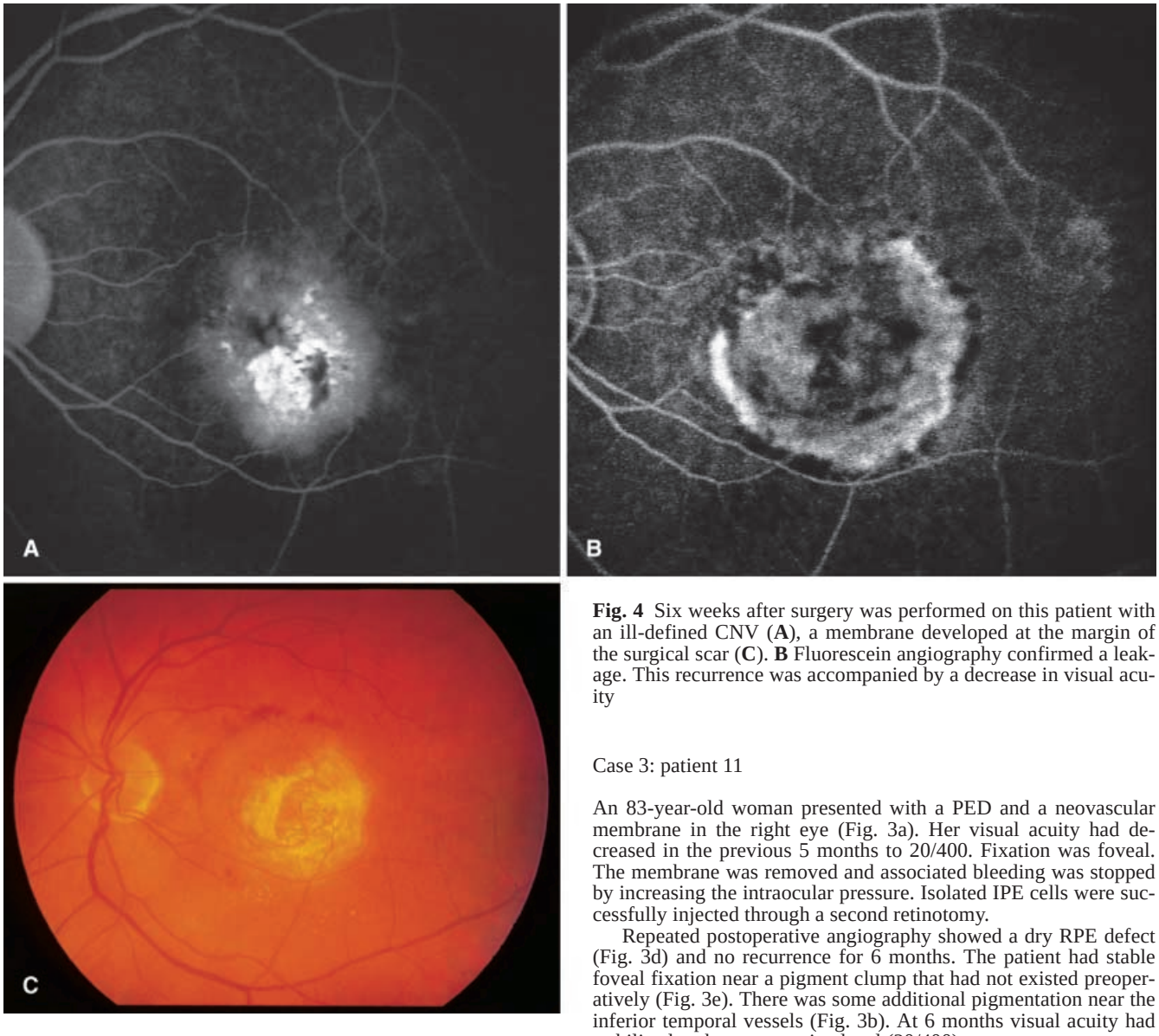

Fig. 4 Six weeks after surgery was performed on this patient with an ill-defined CNV (**A**), a membrane developed at the margin of the surgical scar (**C**). **B** Fluorescein angiography confirmed a leakage. This recurrence was accompanied by a decrease in visual acuity

gressed. No metamorphopsia was noted. Angiography depicted a hyperfluorescent RPE defect without leakage (Fig. 1d). Some additional pigmentation could be discerned within the defective RPE (Fig. 1b).

Case 2: patient 4

A 65-year-old woman presented with a subfoveal ill-defined neovascular membrane of the left eye (Fig. 2a,c). The preoperative visual acuity was 20/100. One month before, she had been able to read. The point of fixation was at the fovea. Intraoperatively, removal of a retinal adherent membrane led to a small retinal hole. The isolated IPE cells were injected subretinally through the retinal hole. Dark pigmented cell clusters were visible under the retina (Fig. 2b). Postoperative visual acuity at 6 months was 20/160, fixation was foveal (Fig. 2e), and angiography showed a dry RPE defect (Fig. 2d).

Case 3: patient 11

An 83-year-old woman presented with a PED and a neovascular membrane in the right eye (Fig. 3a). Her visual acuity had decreased in the previous 5 months to 20/400. Fixation was foveal. The membrane was removed and associated bleeding was stopped by increasing the intraocular pressure. Isolated IPE cells were successfully injected through a second retinotomy.

Repeated postoperative angiography showed a dry RPE defect (Fig. 3d) and no recurrence for 6 months. The patient had stable foveal fixation near a pigment clump that had not existed preoperatively (Fig. 3e). There was some additional pigmentation near the inferior temporal vessels (Fig. 3b). At 6 months visual acuity had stabilized at the preoperative level (20/400).

Case 4: patient 2

One patient showed some bleeding and leakage angiographically after removal of the angiographically confirmed ill-defined CNV. Her visual acuity had decreased in the previous 12 months. Six weeks after surgery, a membrane developed at the margin of the surgical scar (Fig. 4a-c). This was either a recurrent membrane or a persistence; intraoperatively the membrane had been so adherent to the retina that it probably was not removed completely. Visual acuity decreased from 20/80 to 20/250.

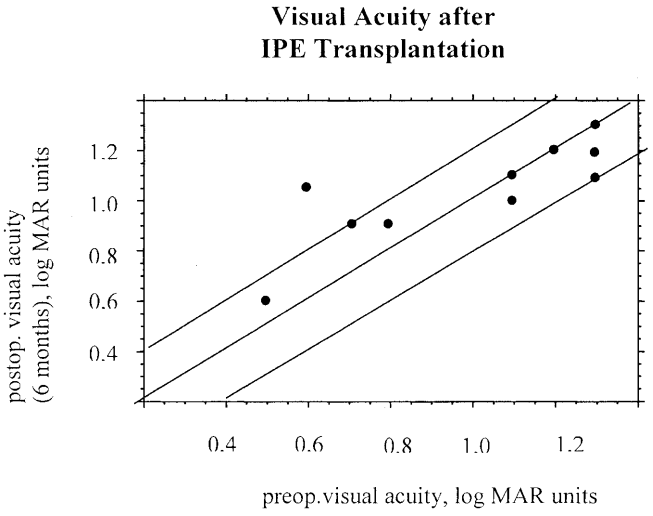

Fig. 5 In this scattergram preoperative visual acuity is shown versus postoperative visual acuity in logMAR units (minimal angle of resolution) A difference of two lines in visual acuity was defined as significant. According to this classification visual acuity remained unchanged in nine patients and decreased in one case

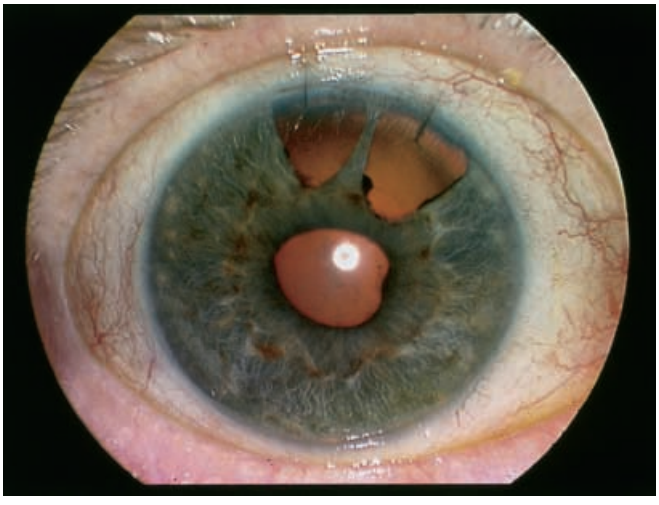

Fig. 6 Two iridectomies were performed to isolate iris pigment epithelial cells

Discussion

This is the first study on IPE cell translocation in patients with ARMD. The injection of whole IPE patches, as reported previously in five patients with ARMD, showed no rejection of the patch [27].

Our study shows that it is surgically feasible to translocate IPE cells beneath the fovea in conjunction with subfoveal CNV removal and to preserve low-level preoperative visual acuity over 6 months.

Visual acuity

Preoperative visual acuity, at a level lower than reading vision, was preserved over a follow-up of 6 months, except for one patient with recurrence. This finding suggests that translocated IPE is functional to some extent, since from the natural course of eyes with subfoveal vascularized PED we expect a decrease in visual acuity of 13%–22% within 4–8 months [37], and in ill-defined CNV we expect a moderate to severe loss of vision in 63% of eyes after 9 months [4]. Besides phagocytosis of photoreceptor outer segments [33, 39] IPE cells synthesize growth factors supporting photoreceptor and choriocapillaris [17]. Such a trophic effect has also been reported for RPE cells. Consequently, fixation within an area of defective RPE without cell translocation/transplantation is more likely in patients with small surgical lesions or after re-epithelialization, as has been observed in monkeys [41]. Repopulation of the postoperative RPE defect, however, may be expected in myopia or POHS more than in ARMD because of senescent RPE cells and compromised adhesion to the Bruch's membrane–choriocapillaris complex [6]. In 6 of 12 patients with IPE translocation, we noted foveal fixation throughout the follow-up. Foveal fixation is an exceptional finding after conventional submacular membrane extraction, even when the size of the choroidal membrane is small, probably due to RPE loss [16, 21, 32].

The mean size of the postoperative RPE defect in patients with extrafoveal fixation was 3.4±0.7 MPS disc areas; in patients with preserved foveal fixation it was 4.3±1.0 MPS disc areas. Therefore, it is unlikely that any trophic effect from RPE cells at the margin of the defect, instead of translocated IPE, is responsible for preservation of foveal fixation.

Three of six patients with retained foveal fixation presented with PED, suggesting preoperative and postoperative persistent interaction between photoreceptors and RPE. Foveal fixation was not preserved in patients who underwent radiation therapy (nos. 1, 3, 7). Consequently good preservation of both photoreceptors and RPE cells may explain in part continued postoperative foveal fixation.

Four of six patients with retained foveal fixation (nos. 2, 4–6) had preoperative visual acuity better than 20/250 and postoperative visual acuity better than 20/200. Good initial visual acuity seems to be beneficial for preservation of foveal fixation and good postoperative visual acuity. Three of seven patients with visual acuity better than 20/250 had an extrafoveal fixation after surgery. They all had a history of radiation therapy (nos. 1, 3, 7). The functional capacity of foveal photoreceptor cells is probably reduced after radiation therapy, and an extrafoveal fixation site at the margin of the RPE defect is preferred. The mean size of the RPE defect after radiation therapy, at 3.7±0.29 MPS disc areas, was not much great-

er than in patients with visual acuity better than 20/250 and foveal fixation (3.3±0.87 MPS disc areas).

We cannot explain how visual acuity in patients with extrafoveal fixation can be better than in patients with foveal fixation. We speculate that factors in addition to visual acuity influence foveal fixation in eyes with low vision.

As to be expected from autologous grafts, we did not note macular edema or pigment clumping typical for graft rejection in homologous fetal RPE transplants [1, 2].

We did not find an increase in visual acuity following IPE translocation, even though foveal fixation was retained. Potential reasons for lack of improvement in central vision are manifold: It is possible that IPE cell reattachment is hampered by age-related changes in Bruch's membrane [6] or by surgically induced defects. We are currently investigating ways of improving IPE cell reattachment, i.e. by co-implantation of basal membrane-like material. Lack of visual improvement could be based on pre-existing photoreceptor and choriocapillaris damage. Compromised photoreceptor layer is known in at least 48% of eyes with subfoveal CNV [13]. Mean preoperative duration of visual loss was 6.6 months. Three of the six patients with extrafoveal fixation had a mean preoperative duration of visual loss of 10,3 months. The remaining three eyes had a history of radiation therapy. Duration of visual loss, however, was difficult to determine as patients do not always immediately perceive the functional deficit when vision is better in the fellow eye. In patients with a long history of visual loss, choroidal membranes strongly adhered to the sensory retina. Removal of those membranes probably caused additional damage to photoreceptors. Also, postvitrectomy cataract formation may have contributed to compromised visual recovery.

Morphology

Postoperative pigmented areas cannot be reliably assigned to IPE. Pigmented cell clumps can be observed after submacular surgery without cell transplantation/translocation. The fact that similar dispersed pigmentation at the posterior pole and in the temporal inferior retinal vascular arcade (sedimented in upright position) were noted at the same time favors the possibility that such pigmentations in fact represented translocated IPE cells. In other eyes, no pigment could be identified at all. Algvere reported a similar observation after RPE transplantation [2]. Possibly some cells died, and single cells were too small to be ophthalmoscopically recognized. We suppose that part of the injected material did not adhere under the macular area but became displaced when the patient took an upright position after surgery.

We could not observe growth of translocated IPE, either because cell proliferation did not occur or because proliferating cells lose their pigment and thereby escape detection. Pigment cell autofluorescence is believed to signal phagocytosis of photoreceptor outer segments [5]. We did not detect autofluorescence in areas of suspected IPE-derived pigmentation. It is unclear whether autofluorescence was not observed because phagocytosis by IPE was absent or because of advanced preexisting retinal degeneration. Perhaps cell seeding was too dispersed for autofluorescence to be detected, or it requires longer than 6 months to become apparent in cells that did not phagocytose beforehand. Recurrence (1 of 12 eyes) was significantly lower than we had expected from the membrane extraction trials [38]. The low rate of recurrence may reflect inhibition by IPE cells. Recurrence always originates from the edge of the RPE defect. It is reported in up to 46% of cases following CNV extraction [38] and in 41% of cases following laser photocoagulation of well-defined CNV [23] after follow-up of 1 year.

Surgical technique

We found that the technique of cell injection into a retinal (macular) bleb using a glass capillary pipette was reliable and straightforward. Our technique of cell injection largely followed the example of Algvere et al. [1, 2]. Spilling of cells into the vitreous cavity was avoided by clogging the retinotomy site with about 0.5 ml of perfluorodecalin. For the cell injection itself, we aimed for the retinal bleb to be as tight as possible, thereby facilitating penetration at its peak by the pointed tip of the micropipette. Transparency of the glass pipette helped to monitor movement of pigment cells. Any subretinal gas bubbles helped to seal the retinal perforation from underneath. Membrane extraction itself sometimes required improvisation due to strong adherence of the CNV to the retina and/or due to bleeding from retinal or choroidal vessels. A complicated membrane extraction was more likely when there was a long duration of visual decrease pre-operatively. Compared to the macular rotation procedures, IPE translocation is still considerably quicker and the surgical risk much lower. The potential for preservation of vision by IPE translocation in ARMD is promising, and deserves further substantiation by improvement of cell reattachment.

References

1. Algvere PV, Berglin L, Gouras P, Sheng Y (1994) Transplantation of fetal retinal pigment epithelium in age-related macular degeneration with subfoveal neovascularization. Graefe's Arch Clin Exp Ophthalmol 232:707–716
2. Algvere PV, Berglin L, Gouras P, Sheng Y, Kopp ED (1997) Transplantation of RPE in age-related macular degeneration: observations in disciform lesions and dry RPE atrophy. Graefe's Arch Clin Exp Ophthalmol 235:149–158
3. Bartz-Schmidt KU, Kociok NGT, Esser P, Heimann K, Schraermeyer U (1999) Detection of mRNA for proteins involved in retinol metabolism in iris pigment epithelium. Invest Ophthalmol Vis Sci 40:1149
4. Bressler NM, Frost LA, Bressler SB, Murphy RP, Fine SL (1988) Natural course of poorly defined choroidal neovascularization associated with macular degeneration. Arch Ophthalmol 106:1537–1542
5. Brunk UT, Wihlmark U, Wrigstad A, Roberg K, Nilsson SE (1995) Accumulation of lipofuscin within retinal pigment epithelial cells results in enhanced sensitivity to photo-oxidation. Gerontology 41[Suppl 2]:201–212
6. Del Priore LV, Tezel TH (1998) Reattachment rate of human retinal pigment epithelium to layers of human Bruch's membrane. Arch Ophthalmol 116:335–341
7. ElDirini AA, Ogden TE, Ryan SJ (1991) Subretinal endophotocoagulation. A new model of subretinal neovascularization in the rabbit. Retina 11:244–249
8. Freddo TF (1984) Intercellular junctions of the iris epithelia in *Macaca mulatta*. Invest Ophthalmol Vis Sci 25:1094–1104
9. Freddo TF (1996) Ultrastructure of the iris. Microsc Res Tech 33:369–389
10. Freddo TF, Raviola G (1982) Freeze fracture analysis of the interendothelial junctions in the blood vessels of the iris in *Macaca mulatta.* Invest Ophthalmol Vis Sci 23:154
11. Gouras P, Algvere P (1996) Retinal cell transplantation in the macula: new techniques. Vision Res 36:4121–4125
12. Gouras P, Berglin L, Sheng Y, Cao H, Lavid J, Lin PK, Bergman L, Kjeldbye H (1995) Long term human RPE transplants in monkey retina. Invest Ophthalmol Vis Sci 36:211
13. Green WR, Enger C (1993) Age-related macular degeneration histopathologic studies. The 1992 Lorenz E Zimmerman Lecture Ophthalmology 100:1519–1535
14. Hu D, McCormick SA, Ritch R (1997) Isolation and culture of iris pigment epithelium from iridectomy specimens of eyes with and without exfoliation syndrome. Arch Ophthalmol 115:89–94
15. Hu DN, Ritch R, McCormick SA, Pelton Henrion K (1992) Isolation and cultivation of iris pigment epithelium. Invest Ophthalmol Vis Sci 33:2443
16. Hudson HL, Frambach DA, Lopez PF (1995) Relation of the functional and structural fundus changes after submacular surgery for neovascular age-related macular degeneration. Br J Ophthalmol 79:417–423
17. Kociok N, Heppehausen H, Schraermeyer U, Esser P, Thumann G, Grisanti S, Heimann K (1998) The mRNA expression of cytokines and their receptors in cultured iris pigment epithelial cells: a comparison with retinal pigment epithelial cells. Exp Eye Res 67:237–250
18. Li LX, Turner JE (1988) Transplantation of retinal pigment epithelial cells to immature and adult rat hosts: short- and long-term survival characteristics. Exp Eye Res 47:771–785
19. Li LX, Sheedlo HJ, Turner JE (1990) Long-term rescue of photoreceptor cells in the retinas of RCS dystrophic rats by RPE transplants. Prog Brain Res 82:179–185
20. Little CW, Castillo B, DiLoreto DA, Cox C, Wyatt J, del Cerro C, del Cerro M, et al. (1996) Transplantation of human fetal retinal pigment epithelium rescues photoreceptor cells from degeneration in the Royal College of Surgeons rat retina. Invest Ophthalmol Vis Sci 37:204–211
21. Loewenstein A, Sunness JS, Bressler NM, Marsh MJ, de Juan E Jr (1998) Scanning laser ophthalmoscope fundus perimetry after surgery for choroidal neovascularization. Am J Ophthalmol 125:657–665
22. Lopez R, Gouras P, Brittis M, Kjeldbye H (1987) Transplantation of cultured rabbit retinal epithelium to rabbit retina using the closed eye method. Invest Ophthalmol Vis Sci 28:1131–1137
23. Macular Photocoagulation Study Group (1991) Laser photocoagulation of subfoveal neovascular lesions in age-related macular degeneration. Results of a randomized clinical trial. Arch Ophthalmol 109:1220–1231
24. Macular Photocoagulation Study Group (1991) Subfoveal neovascular lesions in age-related macular degeneration. Guidelines for evaluation and treatment in the macular photocoagulation study. Arch Ophthalmol 109:1242–1257
25. Macular Photocoagulation Study Group (1993) Five-year follow-up of fellow eyes of patients with age-related macular degeneration and unilateral extrafoveal choroidal neovascularization. Arch Ophthalmol 111:1189–1199
26. Macular Photocoagulation Study Group (1993) Laser photocoagulation of subfoveal neovascular lesions of age-related macular degeneration. Updated findings from two clinical trials. Arch Ophthalmol 111:1200–1209
27. Navea A, Diaz-Llopis MJ, Vilela C, Meng H (1998) Autologous transplantation of iris pigment epithelium into the subretinal space in humans. Invest Ophthalmol Vis Sci 39:97
28. Rezai K, Kohen, L, Wiedemann, P, Heimann, K (1997) Iris pigment epithelium transplantation. Graefe's Arch Clin Exp Ophthalmol 235:558–562
29. Rezai K, Lappas A, Farrokh-Siar L, Kohen L, Wiedemann P, Heimann K (1997) Iris pigment epithelial cells of Long Evans rats demonstrate phagocytic activity. Exp Eye Res 65:23–29
30. Rezai KA, Lappas A, Kohen L, Wiedemann P, Heimann K (1997) Comparison of tight junction permeability for albumin in iris pigment epithelium and retinal pigment epithelium in vitro. Graefe's Arch Clin Exp Ophthalmol 235:48–55
31. Rotmann B, Papermaster BW (1966) Membrane properties of living mammalian cells as studied by enzymatic hydrolysis of fluorogenic esters Proc Natl Acad Sci 55:134
32. Sabates NR, Crane WG, Sabates FN, Schuchard RA, Fletcher DC (1996) Scanning laser ophthalmoscope macular perimetry in the evaluation of submacular surgery. Retina 16:296–304
33. Schraermeyer U, Enzmann V, Kohen L, Addicks K, Wiedemann P, Heimann K (1997) Porcine iris pigment epithelial cells can take up retinal outer segments. Exp Eye Res 65:277–287
34. Schraermeyer U, Kociok N, Heimann K (1999) Rescue effects of IPE transplants in RCS rat: short term results. Invest Ophthalmol Vis Sci :1545–1556

35. Sheedlo HJ, Li L, Turner JE (1989) Photoreceptor cell rescue in the RCS rat by RPE transplantation: a therapeutic approach in a model of inherited retinal dystrophy. Prog Clin Biol Res 314:645–658
36. Silverman MS, Hughes SE (1990) Photoreceptor rescue in the RCS rat without pigment epithelium transplantation. Curr Eye Res 9:183–191
37. Singerman LJ, Stockfish JH (1989) Natural history of subfoveal pigment epithelial detachments associated with subfoveal or unidentifiable choroidal neovascularization complicating age-related macular degeneration. Graefe's Arch Clin Exp Ophthalmol 227:501–507
38. Thomas MA, Dickinson JD, Melberg NS, Ibanez HE, Dhaliwal RS (1994) Visual results after surgical removal of subfoveal choroidal neovascular membranes. Ophthalmology 101:1384–1396
39. Thumann G, Bartz-Schmidt KU, Heimann K, Schraermeyer U (1998) Phagocytosis of rod outer segments by human iris pigment epithelial cells in vitro. Graefe's Arch Clin Exp Ophthalmol 236:753–757
40. Toonen F, Remky A, Janssen V, Wolf S, Reim M (1995) Microperimetry in patients with central serous retinopathy. Ger J Ophthalmol 4:311–314
41. Valentino TL, Kaplan HJ, Del Priore LV, Fang SR, Berger A, Silverman MS (1995) Retinal pigment epithelial repopulation in monkeys after submacular surgery. Arch Ophthalmol 113:932–938