Aim: To evaluate the possibility of translocating autologous peripheral retinal pigment epithelial (RPE) cells and enhance their adhesion to improve functional outcome after choroidal neovascular membrane extraction in patients with subfoveal neovascular membranes.

Methods: A prospective, non-controlled surgical study in eight consecutive patients operated between February and July 2001 with final data monitoring in July 2002. All patients had mixed subfoveal membranes of 2–4 disc diameters. Functional tests included Snellen vision and central fixation testing. During vitrectomy, after the extraction of the neovascular complex, $8 \times 10^6$–$16 \times 10^6$ RPE cells were removed from the periphery and translocated under the macula following the submacular injection of 2 $\mu$g of poly-L-lysine to promote adhesion of the cells.

Results: With a follow up ranging from 3 months to 16 months, a pigmented area was seen in the extraction bed of the neovascular membrane in only one patient. Fixation was at the edge of the extraction bed in three patients. Vision remained the same in five patients and deteriorated in three (all with retinal detachment). Retinal detachment due to proliferative vitreoretinopathy occurred in three patients.

Conclusions: The translocation of autologous peripheral RPE cells after membrane extraction was technically possible in a sterile manner, but was associated with a high proliferative vitreoretinopathy rate and in the present series had no measurable positive effect on functional outcome.

Autologous peripheral retinal pigment epithelium translocation in patients with subfoveal neovascular membranes

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A ge related macular degeneration (ARMD) is the leading cause of visual loss in Europe and North America in patients aged over 75, and in the majority of patients legal blindness is due to choroidal neovascularisation.1 For small subfoveal neovascular membranes laser coagulation was shown to be better than no treatment 2 years after treatment.2 Photodynamic therapy (PDT) has been shown to have better results than controls in patients with a predominantly classic subfoveal membrane.3–4 As there is no evidence based treatment available for large, occult subfoveal neovascularisations, several non-controlled surgical approaches have been reported in such patients. In ARMD malfunction of the retinal pigment epithelium (RPE)-Bruch’s membrane-choriocapillaris complex precedes the loss of macular photoreceptors. In its exudative form, visual loss is accelerated by choroidal neovascular invasion of Bruch’s membrane, under the RPE and into the subretinal space. Surgical removal of such neovascular membranes in patients with ARMD invariably leads to damage of the RPE, as well as the Bruch’s membrane-choriocapillaris complex.5–7 Spontaneous RPE cell repopulation of the damaged area is ineffective or too late, if at all present.8 Consequently, other modifications to recreate the undersurface of the macula have included the injection of a homologous fetal cell patch.9 Its lack of success was partly attributed to a chronic host versus graft rejection. Recently, Weichsel et al10 reported the lack of functional improvement after the injection of HLA matched homologous RPE cells.11 Therefore, several authors used autologous cells: full thickness en bloc translocation of adjacent macular choroid-choriocapillary-Bruch’s membrane and RPE cells—translocation by blunt needle aspiration and reinjection of mid-peripheral RPE cells—as well as the translocation of IPE cells.12 For the last two approaches, however, adherence of the injected cells to the damaged Bruch’s membrane remains uncertain.

We have chosen to translocate peripheral autologous RPE cells. We further tried to enhance RPE cell adherence by the use of poly-L-lysine. Here we report the results of this approach in a case series of eight patients with a subfoveal neovascular membrane.

Patients and methods

Patients with a subfoveal choroidal neovascular membrane that was more than 50% occult on fundus fluorescein angiography (FFA) and larger than 2 disc diameters, with or without submacular blood, were eligible for RPE translocation. Eight patients were included in the study, from 29 January 2001 to 2 July 2001. This study had been approved by the institutional board and written informed consent was obtained from all patients, in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Preoperative examination included history taking, Snellen vision and a dilated pupil fundus examination, including FFA. Postoperative visits were scheduled at 1, 3, and 6 weeks,
3, 6, 9, and 12 months. The final date of monitoring was 30 July 2002. During each visit Snellen vision testing and a comprehensive examination were performed. At 3, 6, and 12 months fundus pictures were taken and central fixation was tested with the smallest spot (500 µm) of the Haag-Straat slit lamp.

Preoperative measures
Three weeks preoperatively argon green laser coagulation retinopexy was performed to wall off the inferior retina by the transretinal injection of Ringer’s solution into the subretinal space. The detached retina was removed up to the ora serrata with the vitrectome. If the patient was phakic, lensectomy was performed at this stage.

Harvesting and analysis of RPE cells
We modified a bent 21 gauge aspiration canula by placing a Nylon 8-0 loop on top and connected it to a 10 ml syringe. The RPE cells were gently scraped off Bruch’s membrane and aspirated through the canula. We collected 20 ml Ringer/RPE aspirate. The cell aspirate was injected into a closed sterile 30 ml centrifuge tube and autologous serum (10%) was added to prevent adherence of the cells to the tubing. After centrifuging (3 g, 5 minutes) within a sterile holder the supernatant was aspirated by the surgeon. The cell pellet was washed once and resuspended in 1 ml of Dulbecco’s modified essential medium (DMEM).

Through a paramacular retinotomy the choroidal membrane was removed from the subretinal space with a subretinal forceps. Poly-I-lysine (0.2 ml, 0.1 mg/ml) was injected under the macula through the existing retinotomy. We tried to minimise reflux through the retinotomy by injecting through a small sclerotomy a 1 ml perflourcarbon (PFCL) bubble over the retinotomy with the injecting canula already inserted under the macula.

After 5 minutes RPE cells were injected subfoveally, again using a PFCL bubble to minimise reflux. Video sequences were made during cell injection, to document proper subretinal injection. Either a 15% mixture of C3F8 gas or a silicone oil tamponade was used and additional laser applied at the peripheral retinectomy site. In gas filled eyes, an iris claw fixated IOL was inserted; in silicone filled eyes an IOL was inserted at the second surgery when we removed silicone oil. The patient was instructed to remain supine as much as possible during the first 24 hours.

Reoperations were performed for retinal detachments or the removal of silicone oil with secondary intraocular lens implantation.

RESULTS
Seven patients had ARMD in both eyes, with a fibrovascular scar in the fellow eye in five patients (table 1). One 37 year old woman had a subfoveal membrane secondary to presumed ocular histoplasmosis syndrome (POHS). The preoperative duration of visual loss in the operated eye ranged from 8 weeks to 6 months. Visual acuity of the operated eye ranged from counting fingers (CF) at 1 metre to 6/120. Preoperative FFAs were obtained in all patients. Membrane size ranged from 2–4 disc diameters. Subretinal blood was present in six patients.

The number of cells analysed per patients varied from $12.5 \times 10^3$ to $70 \times 10^3$. Trypan blue exclusion staining revealed that over 90% of cells were viable.

The heavily pigmented cells we plated adhered to the dish for 5–28 days. Only the cells of patient 4 grew to confluence, with a hexagonal pattern characteristic for RPE cells.

Preoperative course
Subretinal cell injection appeared successful in all patients (also on reviewing the video recordings) except for patient 2 where possible reflux was noticed during cell injection, although at the time we thought this could have been simulated by subretinal blood egressing.

Silicone oil was used as a tamponade in one patient (patient 3), in whom the preoperatively applied laser barrier proved to be ineffective to prevent retinal detachment from the retinectomy site during surgery; a 15% C3F8/air mixture was used in the other seven patients (table 1).

Postoperative course
Retinal detachment developed due to proliferative vitreoretinopathy (PVR) in three patients, who underwent a second vitrectomy with silicone oil tamponade.

Follow up of patients 1 and 3 was only 3 months, because both died. In the other six patients, minimal follow up was 12 months (range 12–16 months). Snellen visual acuity at 3, 6, 9, or 12 months did not show a two line improvement in any patient. In patients 2 and 7 visual loss was clearly caused by the retinal detachment and PVR. These two patients, as well as patient 4, did subjectively worse. Patients 3, 6, and 8 reported some subjective improvement.

Only in patient 8 was a pigmented area noted under the retina within the membranectomy site. A slight increase in pigment was noted during 12 months of follow up; the area of atrophy of choriocapillaris remained unchanged.

Fixation at the edge of the extraction bed of the neovascular membrane was noticed in patients 5, 6, and 8, but not over the pigmented area in patient 8.

In patient 4 (37 year old with POHS) a recurrent submacular haemorrhage was observed ophthalmoscopically after 13 months. No signs of infection were observed in any of the patients.

DISCUSSION
For patients with large, occult subfoveal membranes with or without subretinal blood present, there is no evidence based treatment available. We have translocated autologous peripheral RPE cells to try and improve both the reported autologous RPE21 and IPE20 injection approaches: the latter by choosing RPE cells that may be more likely to take over macular RPE functions than IPE cells21 22; the former by harvesting cells in a manner that would allow us both to collect a greater number of cells and examine these cells for viability in each patient. As to the site of harvesting RPE we chose the inferior retina to minimise the handicap of the resulting scotoma. The disadvantage of having an inferior retinectomy (while the tamponades used had their major effect in the superior half of the retina) was partly overcome by having a formed laser retinopexy at the time of surgery.
We aspirated a fairly large number of cells which we showed in a parallel study to be able to survive the aspiration-centrifuge-injection treatment. On cytospin preparation these cells showed RPE specific staining with cytokeratin 8/18 monoclonal antibodies (van Meurs et al, ARVO abstract 2002, no 683). In that study, however, RPE cells from patients with ARMD (although viable as evaluated by trypan blue exclusion) were unable to grow to confluence.

The number of cells we analysed per patient for quality control varied from 12.5 x 10^4 to 70 x 10^4, but this number of analysed cells was only a small portion of the number of cells actually re-injected. As the average size of the denuded area after the extraction of the choroidal membrane was approximately 4 disc areas with an estimated cell number of 7 x 10^4 RPE cells,21 the transplanted number of RPE cells would be sufficient if not excessive to repopulate the damaged submacular area.

Adhesion of loose clumps of cells to the damaged Bruch’s membrane is important for their survival22 and is likely to be a problem for the cells we and others have injected23 24 25; it is not clear how many of the injected cells managed to settle and function metabolically and elaborate substances to re-establish Bruch’s membrane and modulate choriocapillar growth. We chose to enhance adhesion by the application of a well known laboratory and graft26 adhesion promotor poly-L-lysine. We showed in a parallel study to be able to survive the breakdown. (2) Our technique to try and prevent reflux with aspiration of RPE cells caused a blood-retina barrier was probably brought into contact with the aqueous phase containing an underlayer never occurred; a rapid clearance of the RPE cells by an inflammatory response secondary to the surgical placement technique. Several factors may have contributed to the high incidence related to the translocated cells, but may merely be a reaction of RPE cells by an inflammatory response secondary to the injection site (sealing with PFCL) was probably not adequately closed and adhesion of the RPE cells to the underlayer never occurred; a rapid clearance of the RPE cells by an inflammatory response secondary to the surgical placement technique. Several factors may have contributed to the high incidence related to the translocated cells, but may merely be a reaction to surgical extraction.6 Functionally, vision stabilised in five patients; fixation was on the edge of the extraction bed in three patients, but not over the pigmented area in the only patient with visible pigmentation. In three patients their decrease of vision was caused by retinal detachment.

In conclusion, although we were able to translocate RPE cells in a sterile manner, our findings do not support our assumption that the translocated RPE cells would improve on the functional results of simple membrane extraction. Because of the moderate results we obtained in the patients without complications, as well as the severe complications we encountered in others, we have stopped this approach until we have learned more about means to promote the survival of transplanted RPE cells under the macula.

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