Autologous Transplantation of Retinal Pigment Epithelium–Bruch’s Membrane Complex for Hemorrhagic Age-Related Macular Degeneration

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PURPOSE. To evaluate a surgical procedure for patients with hemorrhagic age-related macular degeneration (AMD).

METHODS. This procedure consisted of excision of the choroidal neovascular membrane and transplantation of autologous retinal pigment epithelium (RPE)–Bruch’s membrane complex. The RPE–Bruch’s membrane complex for transplantation was surgically developed by dissecting Bruch’s membrane with the choriocapillaris from the medium size choroidal vessel layer at the midperipheral region of the choroid. Twenty-one eyes of 21 patients had this surgical procedure. Visual function tests included best corrected visual acuity (BCVA), multifocal (mf)ERG, and microperimetry. Optical coherence tomography (OCT), fluorescein angiography, and autofluorescence examinations were performed to study the status of the transplanted graft.

RESULTS. Among the 21 eyes, 17 with complete clinical data and qualified follow-up durations, which were 20.35 ± 10.31 months on average, were analyzed in this series. On the last follow-up visit, the mean for the ETDRS scores increased from 28.65 ± 25.99 before surgery to 47.76 ± 17.22 after surgery. Microperimetry showed that after surgery, seven eyes gained central fixation at the 12-month follow-up examination. However, two eyes lost their central fixation on the last follow-up visit. Fourteen (82.35%) of the transplanted patches preserved normal color without depigmentation. Among the 21 eyes, proliferative vitreoretinopathy (PVR) occurred in 3 (14.29%), and a recurrent neovascular membrane was observed in one eye (4.76%).

CONCLUSIONS. The transplantation of the autologous RPE–Bruch’s membrane complex can increase the visual acuity of patients with hemorrhagic AMD. The surviving transplanted graft with functional overlying retina was observed after surgery. (Invest Ophthalmol Vis Sci. 2009;50:2975–2981) DOI: 10.1167/iovs.08-2573

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In western countries, age-related macular degeneration (AMD) is the leading cause of blindness in populations older than 55 years.1–8 As life expectancy has increased in recent decades, the number of patients in China with AMD has increased dramatically. Wu et al.9,10 reported that in a survey of 1019 people over 50 years old from the Han ethnic group in China, the prevalence of AMD was 6.4%. Klein et al.11 reported that for the Han ethnic group in the United States, the prevalence of AMD was 4.6%. This report also demonstrated that the prevalence of exudative AMD among Chinese Americans was higher than among African Americans, Hispanic Americans, and Caucasians.11 In elderly people, AMD has become the leading cause of blindness worldwide.

Although CNV accounts for only 10% of total patients with AMD, Ferris et al.12–14 reported that 90% of the severe visual loss among patients with AMD was categorized as CNV, which may present as a massive subretinal hemorrhage. According to several retrospective studies,15–18 if there was no intervening treatment, the prognosis of a massive subretinal hemorrhage would be poor. For a submacular hemorrhage secondary to CNV of AMD, photodynamic therapy (PDT),19–21 photocoagulation therapy,22 and anti-VEGF therapy are not efficient treatments. In eyes with a thick subfoveal CNV, large pigment epithelial detachment (PED), with massive subretinal and/or vitreous hemorrhage, surgery may be necessary to save a patient’s macular visual acuity (VA). Some surgical procedures have been designed to rebuild the normal architecture and function of RPE layer, as it plays a vital role in sustaining photoreceptor functions.23–27 Binder et al.28 reported a controlled clinical trial on transplantation of autologous suspended RPE cells and noted that the patient improved considerably, with no significant difference before and after the transplantation. Stanga et al.29 reported a surgical technique of the RPE layer with full-thickness choroid graft transplantation. All four eyes, however, lost foveal fixation and autofluorescence of the RPE cells on the graft. Later, using a modified technique, several eye centers reported longer lasting results.29–30

We hypothesized that the ideal graft would include the RPE layer with Bruch’s membrane attached by dissecting the choroid from medium-sized choroidal vessel layer, as this might maintain the structural alignment and normal functions of RPE cells and allow the photoreceptors to receive nutrition more efficiently from the underlying choroidal vessels. The similar idea was first put forward by Holz et al.31 and Bindewald et al.32

MATERIALS AND METHODS

Patients

At Peking University Eye Center, 21 patients with hemorrhagic AMD (12 men and 9 women; ages 46–80; average, 69.35 ± 9.46) were initially enrolled in the RPE-Bruch’s membrane complex transplantation study. Ultimately, however, 17 eyes in 17 patients (11 men and 6 women; ages 54–80, average, 68.88 ± 7.36) were included in the
study. Of the initial 21 patients enrolled, 4 were not included because of incomplete clinical data, but the postoperative statuses of those patients are reported later in the article.

Of the 17 patients included in the study, 6 had hemorrhagic CNV with bilateral AMD. Before the surgery, three underwent PDT and two focal photocoagulation therapy. Vitreous hemorrhage was seen in three eyes, and seven presented massive subretinal hemorrhages extending to the temporal vascular arcade on at least one side. As observed in preoperative fundus fluorescein (FFA) and indocyanine green (IGA) angiography, or from the images captured before the massive subretinal hemorrhage occurrence, all 17 eyes demonstrated the occult type of CNV.

Inclusion criteria included: (1) subretinal hemorrhage over at least one blood vessel arch; (2) occult CNV >3.5 DD; (3) area of hemorrhage >50% of the CNV area; (4) best corrected VA better than light perception but worse than 20/80; and (5) duration of massive subretinal or vitreous hemorrhage shorter than 3 months.

Exclusion criteria included (1) systemic disease within the past year, such as a myocardial infarction, which would make the patient an unsuitable candidate for prolonged eye surgery, and (2) CNV with dominant disciform scar.

Before the surgery, all patients were informed of potential risks and possible poor outcomes after the surgery. Written informed consent was obtained from each patient. This study is consistent with the 1964 Declaration of Helsinki and was approved by the ethics committee of the Medical Faculty of Peking University Third Hospital, China.

Surgical Procedure
Before the surgery, 15 of the 17 patients had phacoemulsification. Of the remaining four patients, three had eyes that were pseudophakic, and one had a clear lens. Cataract extraction was not performed on these four patients during the surgical procedure described herein. Closed pars plana vitrectomy (PPV) was performed with four ports. A complete vitrectomy was performed. A hydraulic temporal retinal detachment was developed by subretinal physiology saline injection via a 20-G + 39-G needle (Bausch & Lomb, Tampa, FL). After a semicircular temporal retinotomy just posterior to the ora serrata, the retinal flap was flipped toward the nasal side of the optic disc and fixed by a specially designed pure gold bar with its weight to expose the subretinal space. The gold bar with 2 g weight and 9 mm length was made into 20 gauge, and the bar was tied by a 6-0 surgical suture, the other tip of which was left outside the eye and fixed onto the speculum. Under direct view through a surgical microscope, the perimeter of the CNV membrane was totally exposed after debridement and evacuation of the subretinal blood clot. So that the detached RPE cells around the CNV lesion would be preserved as much as possible, the CNV complex was removed from the underlying choroidal tissue. The bleeding from the feeding choroidal vessel was thoroughly stopped by electric cautery. Then, at the midperiphery of the superior temporal choroidal, electric catarization was performed with an area of 4 mm². A shallow incision was made by a microvitreoretinal (MVR) knife on the catarized spots, and the RPE-Bruch’s membrane complex with attached choriocapillaris was separated from the midsize choroidal vessel layer and uveal stroma. By holding the margin of the separated graft with a forceps, the surgeon could dissect the RPE-Bruch’s membrane complex from the midsize choroidal vessel layer by a specially-designed S-shaped spatula and fine scissors. The graft was transpositioned just at the presumed site of the macula where the CNV membrane was resected. The detached retina was flipped back with perfluorocarbon liquid. Laser coagulation was performed around the retinotomy margin, and silicon oil was injected. Intracocular lenses were implanted in 13 eyes immediately after silicon oil injection. The silicon oil removal was performed approximately 3 months after the initial surgery. All operations were performed by ZM.

Preoperative Examinations
Visual Acuity. Each patient’s VA was tested on an ETDRS chart. The method for measuring VA on the chart was in accordance with the SST (Submacular Surgery Trials) group. Snellen equivalent visual acuity was also tested by the ETDRS chart starting at 4 m. Finger counting was tested from 20 cm and was considered equivalent to 20/3200. An increase of 15 points on the ETDRS chart after surgery was defined as significant improvement of the VA.

Multifocal ERG. The recordings of the mERG, which reflect the summation potentials of cones and cone bipolar cells, were presented in hexagon-related single plots or in three-dimensional photographs. The stimulus array consisted of 103 hexagonal elements. The recorded values were b-wave amplitude intensities (microvolts/square degree) within the central 5° of the retina.

Microperimetry. As microperimetry (Nidek Technologies, Gamagori, Japan) was available in the latter period of this study, the instrument was used to test all the patients after surgery. Fundus images were captured by a real-time infrared camera with a resolution of 768 × 576 pixels. A stimulus was projected to a specific area of the fundus while a retinal image was captured and real-time results were shown on a video monitor. Background illumination was set on 4 apostilbs (asb). Stimulus intensity varied on a one-step scale from 0 to 20 dB, with 0 dB representing 400 asb; the duration of the stimulus was set to 200 ms. A 4-2 Goldmann III strategy was applied to cover 76 spots within 20° of the fundus. Eyes with more than 25% fixation points located within a 4° diameter were defined as eyes with central fixation.

RPE Autofluorescence. By an 488-nm excitation light, 15 consecutive 768 × 768-pixel image were scanned 30° in the central macular area. Then HRA2 software was used to integrate these 15 pictures which could describe the RPE autofluorescence status on a target area.

To evaluate the function and anatomic integrity of the macular area, OCT and angiography (FFA and IGA) were performed on all patients.

Because of a vitreous hemorrhage or massive subretinal hemorrhage, it was difficult to define the perimeter of the CNV membrane for 12 of the 21 eyes. So, to estimate the preoperative macular lesion accurately, we recorded the RPE defect area during the surgery instead.

Statistical Analysis
On the last follow-up visit, paired t-tests of preoperative and postoperative results were compared for ETDRS scores, IOP, and mERG b-wave amplitude with 95% CIs corresponding to the least significant difference. Effectiveness was defined as significant if the probability of the respective tests fell below 0.05. We performed an analysis of a multifactor covariance model, including duration of VA loss, occurrence of vitreous hemorrhage, age, size of the lesion area, occurrence of subretinal hemorrhage over the vessel arch, and preoperative VA. All these clinical data were compared with the frequency of achieving central fixation after surgery. Pearson analysis was used to determine any postoperative relevance between ETDRS scores and central fixation rate.

Results
All clinical data of the 17 eyes shown in Table 1 were from the final follow-up examination with the average follow-up duration of 26.20 ± 9.83 months. After surgery, the average ETDRS scores were significantly improved (47.76 ± 17.22) versus before surgery (28.65 ± 23.99, P = 0.004). Ten patients (10/17) had an increasing VA of more than 13 points after surgery. Five eyes (5/17) showed a change within 13 points (increased or decreased less than 13 points), and two eyes (2/17) showed a decrease of more than 13 points (Table 1, Fig. 1). Snellen equivalent VA results in 10 eyes (10/17) showed an increase in
VA of more than three lines, three eyes (3/17) increased within three lines, two eyes (2/17) decreased less than three lines, and two eyes (2/17) decreased more than three lines. The average IOP was 14.21 \pm 0.40 \text{ mm Hg} before surgery and 14.68 \pm 0.38 \text{ mm Hg} after surgery, both of which were within normal range and with no significant difference ($P = 0.25$).

**Microperimetry**

By microperimetry, 7 of 17 eyes were proved to have central fixation at the 12-month follow-up visit (Figs. 2, 3), whereas two of them (N9, N13) lost central fixation within the next 6 months (Fig. 4).

A multifactor covariance model of the gain of central fixation after surgery showed no statistical relevance among VA loss duration, occurrence of vitreous hemorrhage, age, size of the lesion area, occurrence of subretinal hemorrhage over the vessel arch, and preoperative VA.

After surgery, significant relevance was observed between scores of the ETDRS chart and the central fixation rate by Pearson analysis ($P = 0.002$; Pearson correlation = 0.704).

**Multifocal ERG**

Before surgery, the average amplitude of the b-wave was $52.62 \pm 31.11 \mu \text{V/deg}^2$, and after surgery, it was $67.75 \pm 40.35 \mu \text{V/deg}^2$ with no significant difference ($P = 0.071$).

**Graft Appearance, Angiography, and OCT Examination**

In all 17 eyes, slit-lamp ophthalmic examination showed that 14 grafts presented with normal RPE color. OCT demonstrated that the retinas of the 17 patients had reattached well, but two with macular pucker were observed. Based on late-stage angiography results (FFA and IGA), no leakage was observed from the transplanted area.

**Autofluorescence**

A total of 14 of 17 eyes presented with graft autofluorescence, including 5 with central fixation. And the grafts of 14 eyes with autofluorescence appeared highly consistent with the location of the transplanted RPE. Among the 12 eyes with no central fixation, 7 presented with autofluorescence at the location of RPE graft. In two eyes, no autofluorescence was detected on the grafts. One eye could not be tested due to poor central fixation bilaterally.

**Complications**

In the 17 patients, the identified complications were one recurrent CNV, one graft dislocation, two macular membranes, and one PVR.

As for the four patients who were not included in this series, the first underwent the surgical procedure and acquired neovascular glaucoma. Although his intraocular pressure was back to normal after cryotherapy, his VA remained at hand motions (7 months). Two cases resulted in PVR with retinal detachment, and both of these patients declined to have another operation, with a final VA of 20/200 (5 months) and 20/800 (6 months). The fourth patient had bullous keratopathy.
induced by endothelial decompensation, and an enucleation was performed at a local hospital. Including these four patients, the total complication rate in this study was 42.86% (9/21), with a PVR incidence of 14.29% (3/21).

**DISCUSSION**

The transplantation of the RPE–Bruch’s membrane complex can be divided into three steps:

Step 1: debridement of the extravasated blood both in the vitreous cavity and in the subretinal space by flipping the retina to the nasal side of the optic disc;

Step 2: CNV excision at the macular lesion with maximal preservation of the surrounding normal RPE cells;

Step 3: at the middle peripheral choroidal region, dissection of the transplanting graft from the underlying choroidal tissue.

The two major differences between our surgical procedure and other RPE transplantation surgeries are (1) under the surgeon’s direct observation, the resection of subretinal neovascularization membrane complex was achieved after flipping the overlying retina to the nasal side; and (2) the transplanted graft of RPE–Bruch’s membrane complex with attached choriocapillaris was separated from the medium-size choroidal vessel layer.

By performing the peripheral retinotomy and flipping the detached temporal retina to the nasal side of the optic disc, we exposed the subretinal neovascularization membrane directly to the vitreous cavity. A similar procedure was also used by van Meurs et al. This procedure provided ample space for the retinal surgeon to excise the CNV lesion accurately on the posterior pole with maximum RPE preservation around the lesion. And the gold bar could fix the flipped retina toward the nasal side by its weight to keep a stable environment during CNV excision. A flexible adjustment of the gold bar position could be achieved easily, making it convenient to perform sufficient coagulation on the spot of feeding vessels with direct observation, which could explain the low CNV recurrence (1/21) in our study. In the technique of Stanga et al., excision of the CNV membrane was performed under the

**FIGURE 2.** Case 1 (no. 10). (a, b) Massive subretinal hemorrhage before surgery. In the other eye the diagnosis was long-term vitreous hemorrhage due to AMD. (c) At the 1-year follow-up visit, the transplanted graft showed a normal RPE color on the macular area. (d, f) IGA showed diffused choroidal vessel perfusion underneath the graft and the donor area (white arrow). (e) OCT showed the retina to be well reattached. (g) Stable central fixation was confirmed by microperimetry with sensitive light projection on the graft area while absolute scotoma appeared on the RPE defect area. The numbers surrounding the green cross represent the stimulus intensity. At the 24th follow-up visit, VA was 20/63, The ETDRS score was 74.

induced by endothelial decompensation, and an enucleation was performed at a local hospital.

Including these four patients, the total complication rate in this study was 42.86% (9/21), with a PVR incidence of 14.29% (3/21).

**FIGURE 3.** Case 2 (no. 7). (a, b) The patient underwent PDT twice for subfoveal CNV. There was recurrent CNV and a hemorrhage superotemporal to the original CNV before the surgery. (c) Background fluorescence was shaded by the transplanted graft. (d) The choroidal vessel spread beneath the graft was observed by the IGA examination. (e) OCT showed a flattened graft with tight adhesion of the underlying tissue. (f) Stable central fixation was tested by sensitive light projection on the retina with the graft beneath. (g) The RPE on the graft showed autofluorescence that was highly consistent with normal RPE cells in the peripheral area. BCVA at the month 37 after surgery was 20/50, and the ETDRS score was 74.
In the cases in our study, the CNV lesions attached tightly to the retina when it was flipped over. The increased space combined with the bimanual procedure may avoid creating a macular hole while splitting the adhesion between the CNV membrane and the overlying retina. This explains why there were no iatrogenic macular holes in this study. In Joussen et al., four macular holes developed after surgery in 45 eyes. In our study, because the neural retina of the macular area had been flipped toward the nasal side when the free graft was transpositioned on the presumed subfovea area by perfluorocarbon liquid, damage to the retina was avoided during graft fixation. In Maaijwee et al., patients underwent repeated manipulation during the graft insertion to the subfovea area through a paramacular retinal incision.

In two cases of trauma, we noted that the medium-size choroidal vascular layer could be split (Fig. 5). By experimenting on an enucleated eye, we observed that a regular interface might be created on the outer surface of the choriocapillaris (Figs. 6). Based on earlier observations, we later succeeded in applying this choroidal dissection technique to patients with hemorrhagic AMD. By histopathology, we identified the component of the graft (Figs. 7a, 7b), and the choroidal tissue left behind on the donor area (Fig. 7c) also proved that the dissected graft was split from the medium-size choroidal vascular layer.

Crucial to the success of this surgical procedure, is to find the right dissecting layer of the transplanted graft. The RPE-Bruch’s membrane–choriocapillaris complex in this study included the RPE layer above, the Bruch’s membrane in the middle, and the underlying choriocapillaris. Extrachoroidal tissue was not attached while the choroidal vascular layer was split; a catastrophic hemorrhage can occur during this part of the procedure. Appropriate cautery on the margin of the donor area could not only prevent a hemorrhage when an incision was made with an MVR knife, but also could reduce the blood flow of the choroid within the donor area. If the spatula was in the right layer of the choroid, it was easy to dissect the choroid from the medium-sized choroidal vessel layer, although some small hemorrhages may happen because of complex connections among choroidal vessels.

The advantages of transplanting this complex graft are as follows: (1) The relatively thin graft can be easily managed, spread under the perfluorocarbon liquid without causing any curling, and fixed onto the recipient area without damaging the overlying retina. The absence of rolling edges on the graft margin minimizes the proliferative membrane occurrence and; (2) the thin graft can support the overlying photoreceptors by delivering nutrition from the underlying choroid, which was confirmed by the long-term preservation of central fixation in some patients. We hypothesized that our thinner graft might increase the chance of providing nutrients to the overlying retina sooner. Meanwhile, the technique of full-thickness choroidal graft transplantation results in outcomes similar to ours.

FIGURE 4. Case 3 (no. 13). (a-c) Massive subretinal hemorrhage spread over the vessel arch before surgery. (d) The transplanted graft showed normal RPE color at the 1-year follow-up visit. (e) Leakage of end-stage fluorescein could not be observed from the graft area. Choroidal vessel infusion was observed from the donor area. (f) HE staining of CNV histology (40×) showed the neovascular membrane with dense collagen and RPE on the top, indicating the occult type of CNV. (g) The 95% central fixation located within 4° of the macular area during the microperimetry examination at the 1-year follow-up visit. The ETDRS score at 1-year follow-up was 45. This patient had a recurrent CNV and hemorrhage around the graft at the 15th month after surgery, and the score decreased to 37. VA decreased from 20/200 to 20/500, and central fixation was not preserved.

FIGURE 5. (a) Parallel vessels (black arrow) were observed during the surgery on the posterior interface of the split inner choroidal leaf (open arrow) in an eye injured by trauma. White arrow: margin of the residual outer choroidal leaf. H, the hematoma in the suprachoroidal space. (b) The split choroidal tissue biopsy was obtained from this ruptured eyeball injury. White arrow: the RPE layer; black arrow: medium-sized choroidal vessel; open arrow: choriocapillaris.
It is very important to confirm whether there are viable RPE cells left at the site of the fovea before beginning the transplantation procedure. Accurate evaluation at the time of surgery is necessary to determine the preferred surgical procedure. Options include the following: (1) CNV excision only is performed if the RPE layer is preserved on the subfoveal area; (2) transplantation of the RPE layer is performed only if a naturally detached RPE sheet is available, to avoid sub-RPE hemorrhage; and (3) an RPE–Bruch’s membrane complex with choriocapillaris transplantation is performed if the RPE is absent beneath the fovea and a naturally detached RPE sheet is not available. The first two described surgical procedures were not included in this study.

In this study, microperimetry was an objective method for evaluating the status of central fixation over the graft area. Five of 17 eyes preserved their central fixation on the last follow-up visits with the average follow-up duration of $20.35 \pm 10.31$ months (Table 1). At the month-37 follow-up visit, patient N7 still had central fixation, representing the longest preservation of central fixation in the study (Fig. 3).

Strong correlation existed between central fixation and postoperative ETDRS scores ($P = 0.002$). All five eyes with postoperative central fixation exhibited better than 20/200. The improvement in the ETDRS scores was similar to the result obtained from the Snellen equivalent VA. ETDRS scores emphasized the changes in VA statistically, whereas the Snellen equivalent VA showed the changes in a more common way. The reliability of visual status was enhanced by the use of these two methods of VA recording.

Before and after surgery, mERGs showed that there was no statistically significant difference in the average amplitude of the b-wave. An increased trend, however, was observed after the surgery. The small area of the transplanted graft and the RPE defect area of less than 5° may have compromised the fluctuation of the average b-wave amplitude. Unlike microperimetry, there was no preferred retinal locus between locations of the graft and the high amplitude of mERG hexagonal elements, so mERG results could not provide specific functional evaluation on the retina over the transplanted graft in this study.

Biomicroscopic examination provided another valid assessment of graft survival. All 14 viable grafts showed a healthy RPE brownish color consistent with the autofluorescence observation. Only 5 of the 14 eyes gained central fixation, suggesting that a normal color appearance of the transplanted RPE layer did not correlate with normal neuroretinal function. The other nine eyes, with what appeared to be normal brown color of the RPE layer, showed no central fixation. Although OCT did not show shallow retinal detachment, the sensitivity of the projecting light was not detected by microperimetry on the seemingly well-grafted Bruch’s membrane complex. The possibility of preoperative damage on photoreceptors secondary to massive subretinal hemorrhage should not be ruled out.

PVR as a severe complication with poor prognosis was identified in three cases. The incidence of PVR was 20% to 50% for macular translocation with 360° retinotomy. Three reasons might explain the low PVR occurrence in this study: (1) A 180° retinotomy was performed instead of 360° retinotomy. The temporal retina could be reattached easily to its original position by the attached nasal retina. The retinotomy on the peripheral retina, which had less blood supply and thinner layers, barely needed electrocoagulation. However, retinotomy on the thick posterior pole of retina with rich blood supply might increase the incidence of PVR. The high incidence of PVR in Joussen et al. with 31% PVR occurrence may be related to that reason. (2) The traumatic injury to the central retina was avoided in our study by not performing a paramacular retinotomy, which was a routine procedure in RPE transplantation with full-thickness choroid graft used by many other research groups. (3) The direct observation of the RPE layer provided a clear view for more accurate surgical manipulation, including delamination of the CNV membrane adherent to the fovea, cautering of the CNV feeding vessels, and placement of the free graft onto the recipient area, and it may limit damage of the RPE layer. Compared with the technique of RPE transplantation with full-thickness choroid graft, relatively more complex procedures including 180° retinectomy and choroidal dissection of the free graft could be the reason for higher PVR occurrence, especially in the early stage of the long learning curve. Some steps of the procedure in our study, such as dissection of the graft from the choroid, would be simplified with the development of new surgical tools.

References


