Photoreceptors Repair by Autologous Transplantation of Retinal Pigment Epithelium and Partial-Thickness Choroid Graft in Rabbits

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PURPOSE. To investigate whether autologous retinal pigment epithelium (RPE) and a partial-thickness graft can repair degenerated photoreceptors overlaying a mechanically damaged Bruch’s membrane.

METHODS. Twenty-one pigmented rabbits were used in the study. Abrasive debridement of the RPE was performed with a metal cannula after superior retinal bleb detachment in 20 rabbits. The graft was prepared beneath the inferior retina and was transplanted to the debridement area 14 days later. Debridement-only sites served as the control. Tissue sections were evaluated by light microscopy and transmission electron microscopy at 7 days, 1, 3, and 6 months after transplantation, corresponding to 21, 45, and 90 days after debridement, respectively.

RESULTS. When analyzed at 7 days after transplantation, short buds of inner segment with regularly organized outer nuclear layer were observed. The outer segments (OS) of the graft were of insufficient length to be observed, but by 1 and 3 months, a significant elongation of the OS was detected. In control retinas from 21 days (corresponding to 7 days after transplantation) to 3 months after RPE debridement, the outer nuclear layer cells were disorganized and diminished.

CONCLUSIONS. This study showed that autologous RPE and partial-thickness choroid graft have the capacity not only to support photoreceptor cell survival, but also to initiate early repair mechanisms, as exhibited by outer segment regeneration.

Age-related macular degeneration (AMD) is the leading cause of severe loss of central visual acuity in one or both eyes in people over 50 years of age in western countries and is the most common cause of visual impairment in the elderly Chinese population in Taiwan.1,2 AMD manifests as either an atrophic or an exudative form. In atrophic AMD, geographic atrophy of the retinal pigment epithelium (RPE) and loss of the overlying photoreceptors leads to a decrease in visual acuity.3–6 In exudative AMD, new vessels arising from the choriocapillaris, accompanied by fibroblasts, perforate Bruch’s membrane, resulting in a fibrovascular complex, and invade the space underneath the RPE and/or retina.7–9 Although it is still unknown whether the initial pathologic changes emerge in the RPE or in the choriocapillaris, it is certain that visual loss typically results from photoreceptor degeneration or loss due to underlying RPE atrophy or from destruction by choroidal neovascularization (CNV).10–11

Based on these pathologic changes, a proposed treatment would replace these degenerative RPE cells or CNV membrane with a healthy, functional RPE monolayer that would provide a supportive environment for photoreceptors; however, laser coagulation,12–15 photodynamic therapy,14–16 and antiangiogenic agents17–18 can occlude new vessels, cannot provide healthy RPE,19 and necessitate surgical removal of the CNV membrane.20,21 Transplantation of RPE cells alone has failed to supply functional monolayer RPE cells.22,23 Therefore, autologous transplantation of intact Bruch’s membrane with attached healthy RPE cells from the peripheral retina would be a promising method.

Transplantation of an autologous full-thickness graft consisting of RPE, Bruch’s membrane, and choroid has been shown to promote postoperative visual acuity,24,25 as has transplantation of an autologous partial-thickness graft consisting of RPE, Bruch’s membrane, and the choriocapillaris.26 Survival of a partial-thickness graft is superior to that of a full-thickness graft, and the graft has fewer surgical complications, such as proliferative vitreoretinopathy.20 In addition, protection of overlying photoreceptors has been observed with a partial-thickness graft in a rabbit model.27

In the recent literature, there is no histologic evidence of repair of degenerative photoreceptors by an autologous graft. Herein, we report the anatomic outcome of transplantation of autologous RPE and partial-thickness choroid graft beneath degenerative photoreceptors in rabbits.

MATERIALS AND METHODS

Animals

Twenty-one pigmented rabbits of 1.5 to 2.5 kg body weight were used in our study. Eighteen rabbits were divided into a transplantation group and a control group, with three rabbits examined at three time points in each. Two rabbits were used to establish a baseline (killed at 14 days after debridement), and one additional rabbit served as the normal control.

Twenty rabbits were anesthetized intramuscularly with ketamine (30 mg/kg) and xylazine (5 mg/kg). General anesthesia was maintained by additional ketamine (4 mg/kg) and xylazine (1 mg/kg) every 45 minutes. Pupils were dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride. All animals were cared for in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

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Surgery

RPE Debridement. Three sclerotomies were made 1.5 to 2 mm posterior to the limbus at the inferior, superior temporal, and inferior temporal sclera. The inferior port was used to fix a perfusion cannula. The superior vitreous was removed, and a 20-gauge metal cannula was used to create a retinal detachment in the superior quadrant by slowly injecting balanced saline solution. Subsequently, a microretinotomy was made at the former retinal injection break. Through the retinotomy access, a hollow silicone tip attached to a metal cannula was introduced beneath the detached retina to perform RPE debridement in an area approximately the size of two optic discs, almost one disc diameter from the optic disc. The infusion was performed to maintain the elevation of the detached retina and to avoid destroying the overlying retina. Care was taken to avoid choriocapillaris hemorrhage. The removed RPE was washed away, and fluid was aspirated to flatten the retina.

Graft Transplantations. Fourteen days after RPE debridement, the autologous RPE and partial-thickness choroid graft were transplanted. After a phacoemulsification, three new sclerotomies were made 1.5 to 2 mm posterior to the limbus at the superior temporal, superior nasal, and inferior nasal sclera, respectively. The inferior nasal port was used to fix a perfusion cannula. After complete vitreous removal, a mean 4-optic-disc-diameter rectangular area was surrounded by retinal diathermy coagulation in the inferior retina at least 1.5 disc diameters from the optic disc, and the neural retina was cut. Subsequently, the underlying donor choriocapillaris and the full-thickness choroid were surrounded by choroidal diathermy coagulation. A custom-designed sharp knife was used to perform a gentle break of Bruch’s membrane along one border of the graft, and through the break, a spatula was used to scrape the attached choroid and choriocapillaris as far as possible, until the graft was translucent, and the underlying spatula was visible.

The superior retinal detachment was recreated by subretinal injection of physiologic saline near the former retinal break, a 90° peripheral retinotomy was made to expand the subretinal surgical field, and the graft was incised and transplanted through direct access to the recipient area created 14 days earlier, covering the whole debridement area and some adjacent normal RPE. After transplantation, the retina was reattached with perfluorocarbon (Bausch & Lomb, Waterford, Ireland) followed by fluid–gas exchange. To complete the surgery, the perfluorocarbon was exchanged for silicone oil (Oxane 5700; Bausch & Lomb). Debridement-only animals, serving as the control, underwent complete vitrectomy, and the vitreous cavities were filled with silicone oil.

Tissue Processing

Rabbits were killed by intravenous injection of an overdose of sodium pentobarbital (250–300 mg/kg) at 7 and 14 days, and 1 and 3 months after transplantation, or at the corresponding time point of post-RPE debridement.

For light microscopy, the eyes were enucleated and fixed with saline phosphate-buffered (PBS pH 7.4) 4% paraformaldehyde-2.5% glutaraldehyde. Forty-five minutes later, the anterior segments were removed, and the eye cups were transferred to fresh fixative and stored at 4°C for an additional 24 to 36 hours. The eyes were processed with graded ethanol, and were embedded in paraffin. Sections were cut at a 5-μm thickness, and were stained with hematoxylin-eosin for light microscopy.

For transmission electron microscopy, the eyes were fixed with phosphate-buffered (PB pH 7.4) 2.5% glutaraldehyde overnight. Tissue fragments containing the transplantation site or RPE-removal site were excised and were postfixed in 1% osmium tetroxide and embedded in Epon epoxy resin. The sections were placed on copper grids, stained with uranyl acetate and lead citrate, and photographed with a transmission electron microscope (Tecnai G2 20, Philips, Eindhoven, The Netherlands).

Results

Surgery

In all cases, the retina was reattached within 7 days after RPE removal. During transplantation 14 days after RPE debridement, the debridement sites showed a relatively light appearance, with blood flow in the choroidal vessels through the denuded Bruch’s membrane. The outline of the RPE debridement site showed little hyperpigmentation; however, the area of previous retinal injection appeared darker. The sclera of rabbits is thinner than that of humans, and so we made three new scleral ports during transplantation, lest the ports be enlarged and the graft be discharged in the irrigation fluid during surgery. In three rabbits, choroid hemorrhage occurred at the moment of graft preparation, but ceased by increasing the perfusion pressure and proper choroid diathermy. The hemorrhage did not involve the superior portion of the retinas under which the grafts were transplanted. Prepared free grafts were incurvated to the choroid sides because of collagen-rich tissues in the sides, by which the RPE sides and the choroid sides of grafts could be identified clearly during transplantation. Care was taken to avoid folding the grafts, and the grafts were placed subretinally, flat, and precisely.

Light Microscopy

At 14 days after RPE debridement (Fig. 1B), intact outer segments had disappeared in the retina overlying the debridement sites. Debris materials were observed in the subretinal spaces. Remnants of inner segments and some nuclei of the photoreceptors without inner segments remained intact. No patent choriocapillaries were found beneath the debridement site. At 7 days (Fig. 1B) after transplantation, the vessels in the graft were open. RPE cells on the graft appeared as a simple cuboidal epithelium, resulting from contraction of the graft due to connective tissue on the choroid side, and resulting in the macroscopically dark, hyperpigmented appearance of the

![Figure 1](https://example.com/figure1.jpg)
grafts. Inner segments and barely patent outer segments were observed in the retinas overlying the graft. In the retina 21 days after RPE debridement (Fig. 2D), corresponding to the time point of 7 days after transplantation, the photoreceptors were degenerating (with rarely observed inner segments) or were lost. The outer nuclear layer was attenuated. At 1 month after transplantation (Fig. 2B), the neural retina was regularly organized, and the architecture was normal. Lengthened outer segments were observed. RPE cells on the graft appeared flatter than they had appeared 7 days after transplantation. At 45 days after RPE debridement (Fig. 2E), the retinas had grown thinner, and the photoreceptors were markedly reduced. At 3 months after transplantation (Fig. 2C), the regenerated outer segments were longer when compared with earlier transplantation and appeared to be of approximately mature length when compared with the immediate neighbor. (D) The photoreceptors gradually degenerated with rare inner segments and disappeared. Arrows: fallen RPE cells phagocytosed by microglial cells in the inner retina. (E) The outer nuclear layers were attenuated and had lost normal architecture. (F) Photoreceptors in the retina decreased markedly. The outer nuclear layer was hardly detectable.

Transmission Electron Microscopy

Different stages of the regeneration process in the outer segments were examined by transmission electron microscope. In the retina at 7 days after transplantation, some short buds of the inner segments with a small amount of debris materials were observed directly connecting with the RPE (Fig. 4B). From 1 month to 3 months after transplantation, the inner segments and increasingly mature-appearing, elongated outer segments containing transversely aligned discs connected with RPE on the graft (Figs. 4C, 4D). Functional phagocytotic vesicles were found on the basal side of the RPE. Bruch’s membrane, although it appeared curved because of the contraction of the graft (Fig. 4A), was flattened compared with its earlier appearance. The choriocapillaries were patent and fenestrated.

At 14 days after RPE debridement, the remnant inner segments of degenerative photoreceptors were observed. Significant debris was present between the degenerated photorecep-
tors and the damaged Bruch’s membrane (Fig. 5A). At 3 months after RPE debridement, no normal retina architecture was found. The layers of the retina could not be clearly discriminated. Debris was nonexistent in the subretinal space over the damaged Bruch’s membrane area where the basal lamina of RPE was lost, and the inner collagenous layer was partially damaged (Fig. 5B).

DISCUSSION

This study demonstrates that transplants of autologous RPE and partial-thickness choroid grafts have the capacity to initiate early repair mechanisms, as exhibited by outer segment regeneration in retinas having undergone degeneration of photoreceptors. From 7 days to 3 months after transplantation, outer segments regeneration was observed to progress from a virtual absence of regeneration, to mature tissue, and the retinal architecture overlying the graft had recovered a normal appearance.

In 14-day postdebridement retinas, the outer segments overlying the debridement area had disappeared, and debris material was present at the debridement surfaces, indicating that degeneration had already occurred and that the shed outer segments were beginning to accumulate in the subretinal space. Moreover, the underlying choriocapillaries appeared atrophic, approximately 3 months later, and the outer nuclear layer was extremely short. The abrasive debridement model used in this and other studies has been suggested as a model for macular degeneration because there exists a high correlation between RPE dropout, choriocapillaris atrophy, and loss of photoreceptor function.28 Moreover, Bruch’s membrane was mechanically damaged, although not broken through, which resembles the pathology after CNV excision.29–31
Therefore, the histologic changes in the debridement-only sites in this study are partial evidence that patients experience little visual improvement and that vision may even worsen after CNV excision. In the control group, the debridement sites remained uncovered by RPE, and subsequent death of photoreceptors occurred. The poor repopulation of Bruch’s membrane after abrasive debridement may be the result of damage to Bruch’s membrane, because it is more difficult for the RPE to migrate onto and survive on the basement membrane than to migrate onto and survive on the outer collagenous layer of Bruch’s membrane. Meanwhile, the chondroitin sulfate in Bruch’s membrane may be exposed by abrasive debridement, and a variety of in vitro studies have established an inhibitory role for chondroitin sulfate in axon growth. Together with the loss of RPE, the degeneration and loss of photoreceptors appears to be more extensive in the control group.

In the transplantation group, the vasculature of the graft appeared open, perfused, and viable, as shown by intravascular blood cells, suggesting survival of the autologous grafts on the atrophied choriocapillaris recipient bed, which was apparent as early as 7 days and persisted up to at least 3 months after transplantation. Although the exact timing of revascularization by choroidal neovascular ingrowth of the graft was not examined in our study, the timing of revascularization in skin transplants and in the regeneration of capillaries suggests that the anastomosis of vessels of the graft and the recipient bed may commence within 24 to 48 hours. However, whether the revascularization occurred on the RPE normal recipient bed covered by the graft or directly on the atrophied choriocapillaris recipient bed will be addressed in future studies.

In this study, the continuing elongation of the outer segments partly demonstrates that the autologous RPE and partial-thickness choroid graft rescues the degenerative photoreceptors. Light and electron microscopic observations demonstrated the continuing extension of the outer segments, which corroborated the developmental stages reported for outer segment maturation in the morphogenesis of rod cells of neonatal rats and mice. At 7 days after transplantation (Figs. 2A, 4B), numerous irregularly arranged inner segments extended from the outer nuclear layer. The internal morphology of the inner segments was comparable to that observed in many vertebrate species, and there was less debris materials overlying the graft, which demonstrates the phagocytosis function of RPE on the surviving graft has partial recovered. At 1 month after transplantation, no subretinal debris materials were found over the graft, and outer segment regeneration was observed, but they were much shorter than usual the mature length. By 3 months after transplantation (Figs. 2F, 4D), the photoreceptors appeared to show well-developed outer segments of nearly mature length compared with the retina adjacent to the graft.

The regeneration rate of the outer segments in this study was much slower than the renewal rate of normal outer segments and slower than the regeneration rate of outer segments reattached after retinal detachment. This slow regeneration rate may be explained by the differences in the microenvironments underlying the photoreceptors. In the latter two situations, the outer segments are nourished by RPE overlying healthy choroid circulation; however, in our study, the outer segments attached to the RPE on the free graft, and the graft lies on the atrophic choriocapillaris recipient site. As we have learned from experience with free grafts in skin transplants, the time required for a graft to progress from revascularization to complete recovery of sympathetic nerve innervation is 4 to 8 weeks. Therefore, we could speculate that complete functional recovery of RPE on the graft would take some time, and consequently, the regeneration rate of outer segments would be relatively slow.

The graft transplantations were performed 14 days after RPE debridement in this study, and the transplantation opportunity was chosen by the histologic changes in retinas subjected to RPE debridement in our previous studies and earlier literature. Inner segments remain at 14 days after debridement, and giant mitochondria and Golgi bodies in the inner segment ellipsoids are necessary for regeneration of the outer segments. However, whether the histologic changes at 14 days after debridement are the same as those that would occur when autologous transplantation is clinically applied in a patient with AMD is not yet clear, and no quantification of photoreceptor death and corresponding electroretinography was performed in this study. The latest time at which surgery could rescue the dying photoreceptors would be evaluated in further studies.

In two cases in this study, the photoreceptors were partially repaired. The remaining degenerating photoreceptors located overlying the graft free of RPE which would fall off or be destroyed by intraocular forces during the transplantation. The degeneration process in these areas resembled that of the RPE-debridement sites. When degenerating RPE cells fell onto their adjacent locations and nourished the overlying photoreceptors, even the ones overlying the RPE-free sites exhibited the “attraction” of the outer segments and the photoreceptor nuclei (Fig. 3A). This directly confirms the previous idea that the RPE plays a key role in the maturation of outer segments. Furthermore, we suppose that the growth factors secreted by RPE cells would function as chemokines that stimulate the
migration of photoreceptors as occurs in normal development.\textsuperscript{40,41}

When the transplantation was performed, no obvious fibrous adhesion was observed between the previously detached retina and the underlying tissues, and slightly darker pigmentation was observed along the edge in the RPE debridement area. These results differ from those of a previous study,\textsuperscript{42} and the difference has two possible explanations: first, that the proper reattachment of the neuronal retina could inhibit the transformation from RPE cells to fibroblasts and inhibit the proliferation of RPE cells and glial cells;\textsuperscript{43} and second, that although Bruch’s membrane was mechanically injured, no choriocapillaris hemorrhage was seen in the debridement area, and thus, would not mediate inflammatory or fibrous adhesions.\textsuperscript{44} However, the pigmentation along the edge of the previous retinal injection break was prominent, and fibrosis occurred in a part of the break, which resembles the conditions in the previous studies in which the RPE was damaged with removal of the overlying neuronal retina followed by injury to the choriocapillaris.\textsuperscript{42}

In conclusion, the results of this study have shown that autologous RPE and choriocapillaris graft transplantation have the capacity not only to support photoreceptor survival, but also to initiate early repair mechanisms exhibited by outer segment regeneration in the degenerative retina overlying the graft. Therefore, autologous RPE and partial-thickness choroid graft transplantation may offer a means to treat severe subretinal hemorrhage in exudative AMD or geographic atrophy.

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**References**