Graefe's Arch Clin Exp Ophthalmol (1999)
237:668–677 © Springer-Verlag 1999

LABORATORY INVESTIGATION

Atsushi Hayashi
Ajit B. Majji
Sayuri Fujioka
Hyung Chan Kim
Ichiro Fukushima
Eugene de Juan Jr.

Surgically induced degeneration and regeneration of the choriocapillaris in rabbit

Received: 1 September 1998
Revised version received: 4 November 1998
Accepted: 5 November 1998

A. Hayashi · A. B. Majji
S. Fujioka · H. C. Kim · I. Fukushima
E. de Juan Jr. (✉)
Wilmer Ophthalmological Institute,
The Johns Hopkins University,
School of Medicine, Maumenee 738,
600 N. Wolfe St., Baltimore, MD 21287,
USA
Tel. +1-410-550-5383,
Fax +1-410-614-9315

Abstract ● Background: Retinal pigment epithelium (RPE) and choriocapillaris play an important role in maintaining the outer retina. Clinical and experimental studies have shown that there is a close relationship between them. To examine the relationship between RPE and choriocapillaris we developed an animal model in which degeneration and regeneration of the choriocapillaris can be created easily and reproducibly. ● Methods: Using pigmented rabbits a retinal detachment (about 7 disk diameters) was created in the eye and the detached retina was surgically removed. A half area of the exposed RPE was mechanically debrided using a silicone brush. The other half area remained untouched. The eyes were morphologically examined at 3, 7, and 14 days and 4 and 8 weeks after the surgery using scanning or transmission electron microscopy. Changes in the choriocapillaris after the RPE debridement were also evaluated by corrosion vascular casts. ● Results: The debrided area was resurfaced with elongated RPE in 7 days after surgery. The replaced RPE was dedifferentiated but it gradually recovered cell polarity by 4 weeks after surgery. The choriocapillaris beneath the debrided area underwent degeneration; however, it regenerated by 4 weeks after surgery, corresponding to the timing of the morphologic recovery of the replaced RPE. ● Conclusion: This animal model of surgically induced degeneration and regeneration of the choriocapillaris may be useful to clarify the relationship between RPE and choriocapillaris and to study potential treatments for choroidal vascular diseases.

Introduction

The choriocapillaris is located adjacent to retinal pigment epithelium (RPE) and Bruch's membrane in the eye and provides vascular support to the outer retina [8]. Atrophy of the choriocapillaris, which results in poor visual outcome, is often accompanied by age-related macular degeneration, retinitis pigmentosa, and surgical removal of choroidal neovascular membranes in exudative age-related macular degeneration, presumed ocular histoplasmosis syndrome, and high myopia [7, 9, 11, 12, 27, 28, 30, 34, 36, 39].

The relationship between RPE and choriocapillaris has been studied by several investigators [6, 13, 17–20, 22]. Experimental studies of selective destruction of the RPE by administration of sodium iodate or mechanical debridement proved that degeneration or removal of RPE caused atrophy of the choriocapillaris [13, 14, 19]. This led investigators to postulate that vascular modulating factors are released from the RPE [13, 18, 19]. In addition, the Royal College of Surgeons rat with hereditary retinal degeneration has been shown to display loss of choriocapillaris [4, 25]. On the other hand, previous studies regarding regeneration of the RPE after laser burns, administration of sodium iodate, and debridement have shown that regeneration of the RPE induces regeneration and differentiation of the choriocapillaris [3, 21, 25, 31, 32].

However, the mechanisms by which the RPE regulates and maintains the choriocapillaris are not well understood. Recent studies have shown that several factors act as survival and differentiating factors for vascular endothelial cells in vitro and in tumors such as basic fibroblast growth factor, vascular endothelial growth factor, and extracellular matrix [1, 2, 16, 23, 33, 35, 38]. To investigate in vivo mechanisms of regulation of choriocapillaris by RPE it is necessary to develop an animal model in which degeneration and regeneration of the choriocapillaris can be easily created and controlled. In this study we have created a model of degeneration and regeneration of the choriocapillaris in rabbit by a surgical debridement of RPE to explore future treatments directed at atrophy of the choriocapillaris accompanied by vision-threatening diseases.

Materials and methods

RPE debridement surgery in rabbits

Sixty male pigmented rabbits (2–2.5 kg body weight) were used in this study. Rabbits were anesthetized with intramuscular injection of ketamine (30 mg/kg) and xylazine (5 mg/kg) and the pupil was dilated with topical application of 1% tropicamide and 2.5% phenylephrine. Topical 1% atropine was also applied to the operated eye before and after the surgery to reduce post-surgical inflammation. Only one eye of each animal was operated upon. Topical 0.5% proparacaine hydrochloride was used for local anesthesia of the eye surface.

Surgical procedures were modified from a previous study by Heriot and Machemer [14]. Two areas of the limbal conjunctiva was cut for 60 deg each and the sclera was exposed. Then, two scleral mattress sutures were placed at 1.5 mm peripheral from the limbus in the superotemporal and superonasal quadrants with 8–0 silk. Sclerotomy sites were created on these sites with a 22-gauge needle. The superotemporal site was used for intraocular infusion of lactated Ringer's solution, and the superonasal site for introduction of vitreous cutter and needles. A contact lens was placed on the cornea, and the formed vitreous above the inferior retina was removed with a vitreous cutter using the coaxial illumination of an operating microscope. Then, a 30-gauge blunt needle attached to a syringe filled with lactated Ringer's solution was introduced through the sclerotomy site and placed just above the retina 2 disc diameters inferior to the optic disc. A jet stream from the 30-gauge needle was blown against the retina and the retina was detached after formation of a small retinal break. With continuing injection of lactated Ringer's solution into the subretinal space the retinal detachment was extended to a size of about 7 disc diameters. Then, the detached retina was removed with a vitreous cutter and the RPE was exposed to the vitreous cavity. Half of the exposed RPE (right side or left side) was removed from Bruch's membrane with a fine silicone brush and the other half of the RPE remained untouched. The removed RPE was suctioned away with a vitreous cutter and the sclerotomy sites were closed. After surgery gentamicin and prednisolone ointment were placed in the cul-de-sac.

The operated eyes were observed by indirect ophthalmoscopy at 3, 7, and 14 days and 4 and 8 weeks after the surgery. Fundus photography and fluorescein angiography were performed (Topcon, Tokyo, Japan) at these time points. All procedures involving the animals conformed to the ARVO *Statement for the Use of Animals in Ophthalmic and Vision Research*.

Histologic preparation

Under deep anesthesia, 30 rabbits were killed by an overdose injection of pentobarbital for histologic examinations (5 rabbits each at 3 h, 3, 7, and 14 days, 4 and 8 weeks after the surgery). After a small incision was made at the limbus, the eyes were fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer overnight. Anterior portions were removed and posterior eye cups were prepared. The surgical wound in each eye, which included the area of the debrided RPE and the area of the untouched RPE, was cut into halves with a surgical blade so that each half included a half area of the debrided RPE and a half area of the untouched RPE. One half was processed for transmission electron microscopy to examine ultrastructure of the RPE and the choriocapillaris and the other for scanning electron microscopy to examine surface appearances of the healing RPE.

Transmission electron microscopy

A half wound area in each eye was further cut into small pieces and postfixed in 1% osmium tetroxide. After they were rinsed in 0.1 M phosphate buffer, the pieces were dehydrated through a graded series of ethanol. After being placed in two changes of propylene oxide for 15 min each and infiltrated overnight in a 1:1 solution of propylene oxide and LX112 resin (Ladd Research Industries, Burlington, Vt.), the pieces were embedded in 100% LX112 resin. Sections were cut into 1-µm slices and stained with 0.5% toluidine blue and 0.25% sodium borate. Then, specific areas in the sections were cut into 60- to 90-nm slices with an ultramicrotome and picked up on 200-mesh copper grids. The sections were stained with 2% aqueous uranyl acetate for 10 min followed by 3–4 min in Reynolds lead citrate solution. The sections on grids were observed under a JOEL 100 CX transmission electron microscope (Hitachi, Tokyo, Japan).

Scanning electron microscopy

The other half of the wound area in each eye was rinsed with 0.1 M phosphate buffer and then dehydrated through a graded series of ethanol. Then, the tissues were dried to the critical point with tetramethylsilane (Ted Pella, Redding, Calif.). After the tissues had been sputter-coated with gold-palladium, they were examined with a JOELJSM 840 scanning electron microscope (Hitachi).

Preparation of corrosion cast of choroidal vasculature

After the rabbits had undergone surgery as described above, another group of 30 rabbits were deeply anesthetized, killed and processed for corrosion vascular casts at 3 h, 3, 7, and 14 days, and 4 and 8 weeks after the surgery (five rabbits each at these time points). Procedures for preparation of corrosion vascular cast were reported previously [10]. Briefly, the skin of the neck was cut along the trachea and opened carefully with forceps. Then, carotid arteries and veins on both sides were exposed. The carotid arteries were tightened with 4–0 silk sutures. A 22-gauge intravenous catheter was inserted into each carotid artery and the carotid veins were opened. Blood in the head was washed out with 500 ml of heparinized lactated Ringer's solution from both carotid arteries. After the blood was completely flushed out, 50 ml of red Mercox mixed with 1.1 g of the organic peroxide catalyst (Ladd Research Industries) filled in a 60-ml syringe was injected through each carotid artery simultaneously. After hardening of the Mercox for 60 min, the eyes were enucleated and the anterior portions removed. Eye cups were immersed in 100 ml of 1 M potassium hydroxide in distilled water, changed several times at room temperature until all tissues were completely digested. Usually, in 7–10 days only corrosion vascular

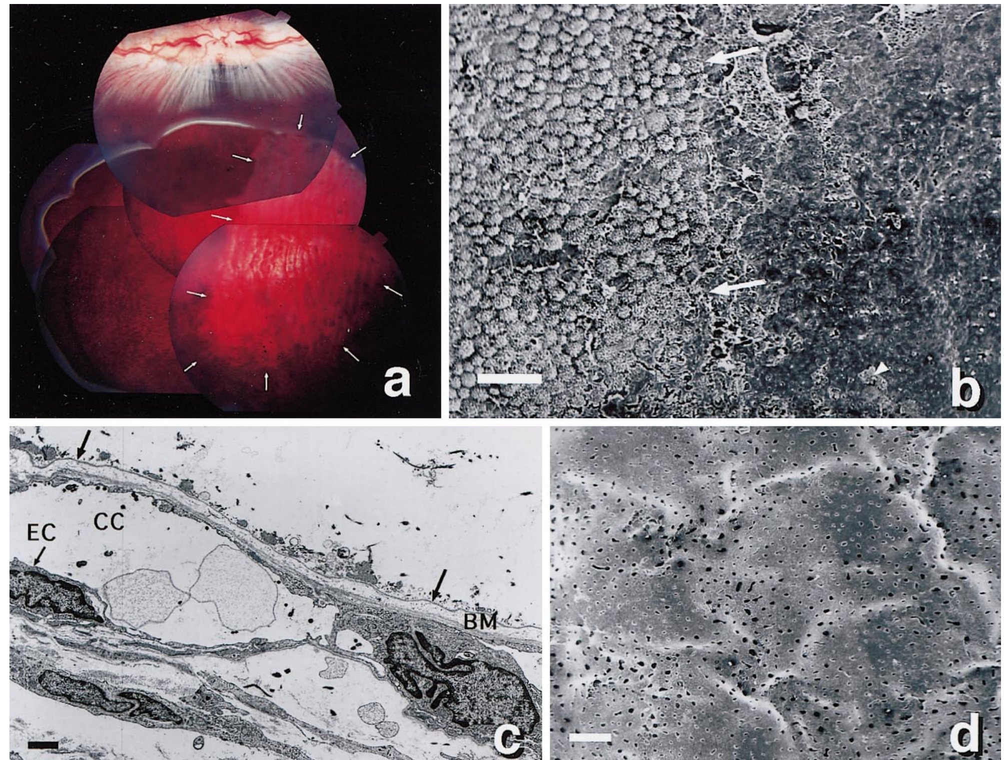

Fig. 1a–d Clinical appearance and histologic observations of rabbit eyes at 3 h after the surgery of debridement of RPE. **a** A fundus color photograph. The area enclosed with *arrows* shows the debrided area of the RPE where choroidal vessels are visible. **b** A photomicrograph of the RPE and Bruch's membrane at the border of the debrided RPE by scanning electron microscopy. The demarcation line is obvious (*arrows*). *Bar* 50 μm. **c** A photomicrograph of the debrided area by transmission electron microscopy. *Arrows* Basement membrane of RPE, *BM* Bruch's membrane, *CC* choriocapillaris, *EC* endothelial cell. *Bar* 1 μm. **d** A photomicrograph of corrosion vascular cast in the debrided area, which indicates normal appearance of the choriocapillaris. *Bar* 50 μm

casts of the retina and the choroid were left in the 1 M potassium hydroxide solution. Vascular casts were rinsed in distilled water several times. After the retinal vessels were removed, the vascular casts were dried in air. After the casts were mounted and sputter-coated with gold-palladium, they were observed with a JEOL JSM 840 scanning electron microscope. Each cast had a defect of the choroidal vasculature at the optic disc and this could be used for orientation. To quantify degeneration of the choriocapillaris, three photographs were taken in three different areas in the center of the degenerated choriocapillaris of each vascular cast. Each photograph represented an area of 0.39 mm^2 in the vascular cast.

The photographs were scanned and image-analyzed by Adobe Photoshop (version 4.0) (Adobe Systems, San Jose, Calif.) in a Macintosh computer. Since the choriocapillaris appeared white with an intense contrast to the background in each photograph, a threshold was set in the histogram of each image to dissect out the white vascular area. The total area of the choriocapillaris was obtained by measuring the area over the threshold in each image. Although larger choroidal vessels under the choriocapillaris appeared darker, some of them were included in the total vascular area when the choriocapillaris was degenerated. However, the majority of the measured area in each photograph represented the choriocapillaris.

Results

Clinical and histologic observation just after surgery

A fundus photograph at 3 h after the surgery is shown in Fig. 1a. The detached retina about 7 disk diameters had been removed and a half area of the exposed RPE debrided. This was recognized as a red area with the blood flow in choroidal vessels through the exposed Bruch's membrane (enclosed with arrows). The other half appeared darker because of melanin pigments in the untouched RPE and the demarcation line was obvious (Fig. 1a). Debrided areas in other eyes were examined by scanning or transmission electron microscopy. Figure 1b shows an area around the demarcation line by scanning electron microscopy in which the RPE was completely removed in the debrided area. Arrows show the demarcation line. Cellular debris was seen on the exposed Bruch's membrane (arrowhead). Figure 1c is a transmission electron microscopic photomicrograph which shows that the basement membrane of RPE in Bruch's membrane was continuous (arrows). Endothelial cells of the choriocapillaris appeared normal (Fig. 1c). The choriocapillaris in the debrided area as examined by vascular casts at 3 h after surgery appeared normal (Fig. 1d). These results were confirmed in all eyes examined at 3 h after surgery.

Clinical appearance after surgery

The same eye shown in Fig. 1a was followed at 3, 7, and 14 days and 4 weeks after surgery by fundus photography and fluorescein angiography (FA). At 3 days after surgery choroidal vessels were visible in the debrided area, while some RPE in the untouched area had lost its pigmentation (Fig. 2a). The demarcation line was visible in a fundus photograph (arrows in Fig. 2a). FA showed extensive

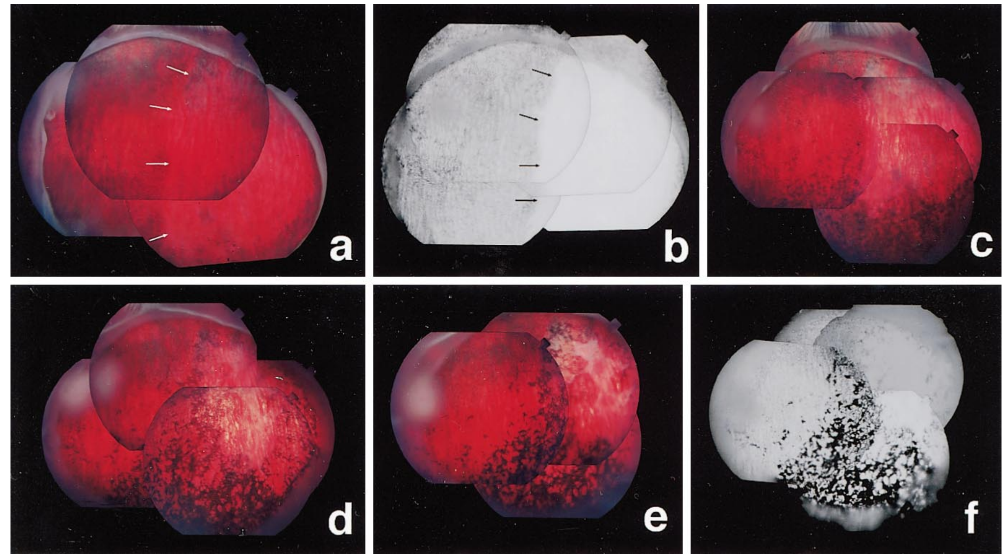

Fig. 2a–f Fundus photographs and fluorescein angiographs (FA) of the eye after the surgery of debridement of RPE. The same eye shown in Fig. 1a was followed up at 3, 7, and 14 days and 4 weeks after surgery. **a, b** Fundus color photograph and FA 3 days after surgery. **c, d** Fundus color photographs 7 and 14 days after surgery, respectively. **e, f** Fundus color photograph and FA at 4 weeks after surgery

leakage of the fluorescein from the debrided area although the untouched area did not show any leakage (Fig. 2b). The demarcation line was more apparent on FA (arrows in Fig. 2b). At 7 days after surgery the center of the debrided area appeared a little whiter, probably because of some loss of the choriocapillaris (Fig. 2c). Along the edge of the debrided area darker pigmentation in the RPE was observed (Fig. 2c). FA showed decreased leakage and a window defect of the fluorescein in the debrided area at 7 days after surgery (not shown). At 14 days after surgery the pigmentation along the edge was more prominent and fibrosis occurred in a part of the debrided area. Large choroidal vessels were more visible in the debrided area, which implied atrophy of the choriocapillaris (Fig. 2d). FA at 14 days after the surgery showed the same appearance as at 7 days after surgery (not shown). At 4 weeks after surgery heavy pigmentation was observed along the wound edge, but other features were similar to those at 14 days after surgery (Fig. 2e). FA at 4 weeks after surgery showed hyperfluorescence by window defect and tissue staining in the debrided area and hypofluorescence at the highly pigmented foci (Fig. 2f).

All eyes examined at 8 weeks after surgery showed the same fundus and FA appearances as at 4 weeks after surgery.

Correlation between healing of RPE and degeneration of choriocapillaris

Using the technique of corrosion vascular cast, the choriocapillaris beneath the surgically debrided area was proved to be degenerated after surgery. The degenerated choriocapillaris was apparent at 3 days after surgery (enclosed with arrows in Fig. 3a). A magnified photomicrograph showed a clear demarcation line dividing the degenerated choriocapillaris from the normal choriocapillaris (arrows in Fig. 3b). Although the side and area of the degenerated choriocapillaris in each vascular cast always corresponded to the debrided side and area of the RPE, this demarcation line was not proved to correspond exactly to the demarcation line observed in fundus photographs because tissues had been already digested to prepare vascular casts. Figure 3c, a magnified photomicrograph of the degenerated choriocapillaris in Fig. 3b, shows that total area of perfused lumina of the choriocapillaris was decreased. The choriocapillaris beneath the exposed and untouched RPE had a normal appearance at 3 days after surgery (Fig. 3d). All vascular casts after the surgery showed similar degeneration of the choriocapillaris beneath the debrided area, but the choriocapillaris was normal beneath the untouched area with a clear demarcation line.

To examine a correlation between wound healing of the RPE and changes in the choriocapillaris, we observed both the RPE in the center of the debrided area and the choriocapillaris in the center of the degenerated area in each vascular cast. At 7 days after the surgery the debrided area was resurfaced with elongated RPE, as shown in Fig. 4a. However, cell borders of the resurfaced RPE were not obvious and cell sizes were varied (Fig. 4a). Vascular casts showed that degeneration of the choriocapillaris had progressed compared with 3 days after surgery (see Fig. 3c). Large choroidal vessels were seen through the degenerated choriocapillaris (Fig. 4b). At 14 days after surgery the elongated RPE cells were becoming polygonal and some cell borders were seen, with appearance of smaller cells (Fig. 4c). In vascular casts, severe degeneration of the choriocapillaris was still observed and was similar to that at 7 days after surgery (Fig. 4d). At 4 weeks after surgery the resurfaced RPE showed a more polygo-

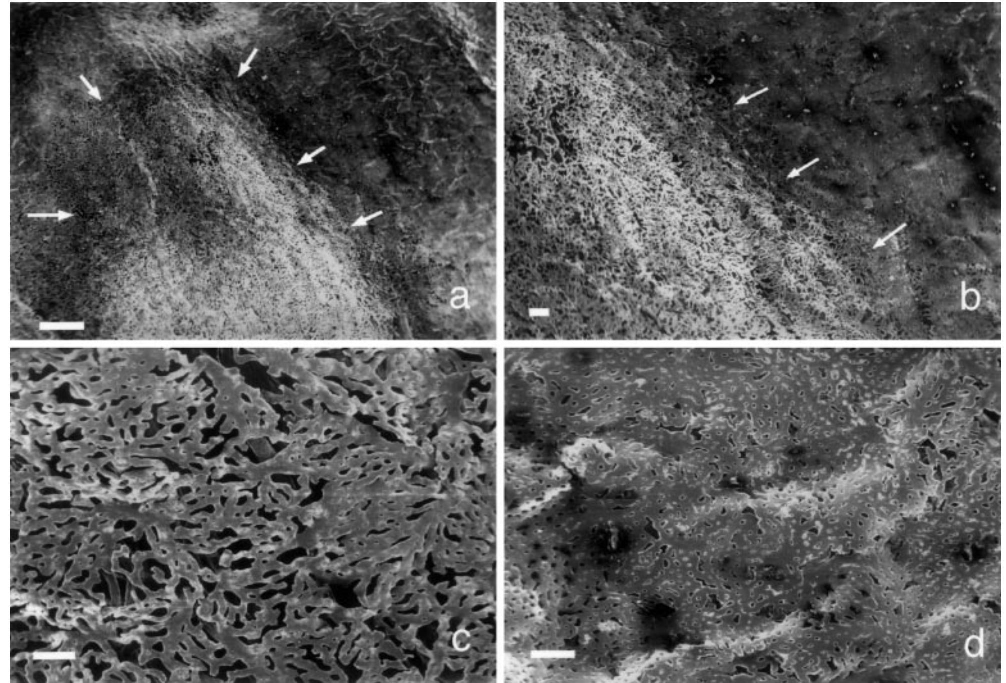

Fig. 3a–d Photomicrographs of corrosion vascular casts at 3 days after the surgery. **a, b** Low-magnification photomicrographs of vascular casts show a clear demarcation line between the degenerated area and the normal area (*arrows*). *Bars*: **a** 500 µm, **b** 100 µm. **c** A magnified photomicrograph of the vascular cast at the center of the degenerated choriocapillaris. *Bar* 50 µm. **d** A magnified photomicrograph of the vascular cast at the center of the exposed and untouched RPE. *Bar* 50 µm

nal shape and small cells appeared with microvilli on the apical surface (arrows in Fig. 4e), with visible cell borders. However, some parts of the RPE were still elongated and multi-layered (Fig. 4e). Vascular casts showed slight recovery of the choriocapillaris. Lobule structures of the choriocapillaris were recognized (arrows in Fig. 4f). At 8 weeks after surgery the RPE had recovered a polygonal shape and appeared homogeneous. More RPE cells had microvilli on the apical surface with apparent cell borders (arrows in Fig. 4g). Vascular casts showed regeneration of the choriocapillaris compared with 4 weeks after surgery (Fig. 4h).

Since the choriocapillaris beneath the untouched RPE without the overlying retina showed a normal appearance until 8 weeks after surgery, the surgical trauma itself and the absence of the overlying retina did not induce degeneration of the choriocapillaris.

Quantification of choriocapillaris after surgery

Changes in the choriocapillaris after surgery were evaluated by measuring the relative area of the choriocapillaris in each photomicrograph using image analysis software (Adobe Photoshop). One mean value was obtained from three photomicrographs taken in the center of the degenerated choriocapillaris in each vascular cast. Five different vascular casts at each time point were examined, and the mean relative area of the choriocapillaris in each photomicrograph was expressed as a percentage of that for the normal choriocapillaris. The mean relative area of the choriocapillaris at 3 days after the surgery was 78.8±5.4% (mean ± standard deviation). The mean relative areas of the choriocapillaris at 7 and 14 days and 4 and 8 weeks after surgery were 60.7±6.3%, 57.1±1.0%, 60.1±1.5%, and 74.6±4.6%, respectively. The choriocapillaris degeneration progressed until 14 days after surgery and from 4 weeks after surgery it gradually regenerated.

Ultrastructural changes in the RPE and the choriocapillaris

Ultrastructural changes in the RPE and the choriocapillaris in the debrided areas were examined by transmission electron microscopy. A photomicrograph of a normal rabbit eye was shown in Fig. 5a. The lumen of the choriocapillaris was patent and endothelial cells of the choriocapillaris were normal. Fenestrations of the endothelial cells were apparent only on the side of Bruch's membrane (arrowheads in Fig. 5a). RPE showed its cell polarity with apical microvilli, junctional complex, and basal infoldings with mitochondria at the basal side and melanin granules at the apical side in the cytoplasm (Fig. 5a). At 7 days after surgery, as shown in Fig. 5b, the debrided area was resurfaced with elongated RPE. Some resurfaced RPE cells were piled up and multi-layered. Those RPE cells appeared dedifferentiated with scattered melanin granules in their cytoplasm, microvilli on both sides of the cell surface, and reduced basal infolding. The lumen of the choriocapillaris was filled with necrotic debris and the endothelial cells of the choriocapillaris showed pyknotic

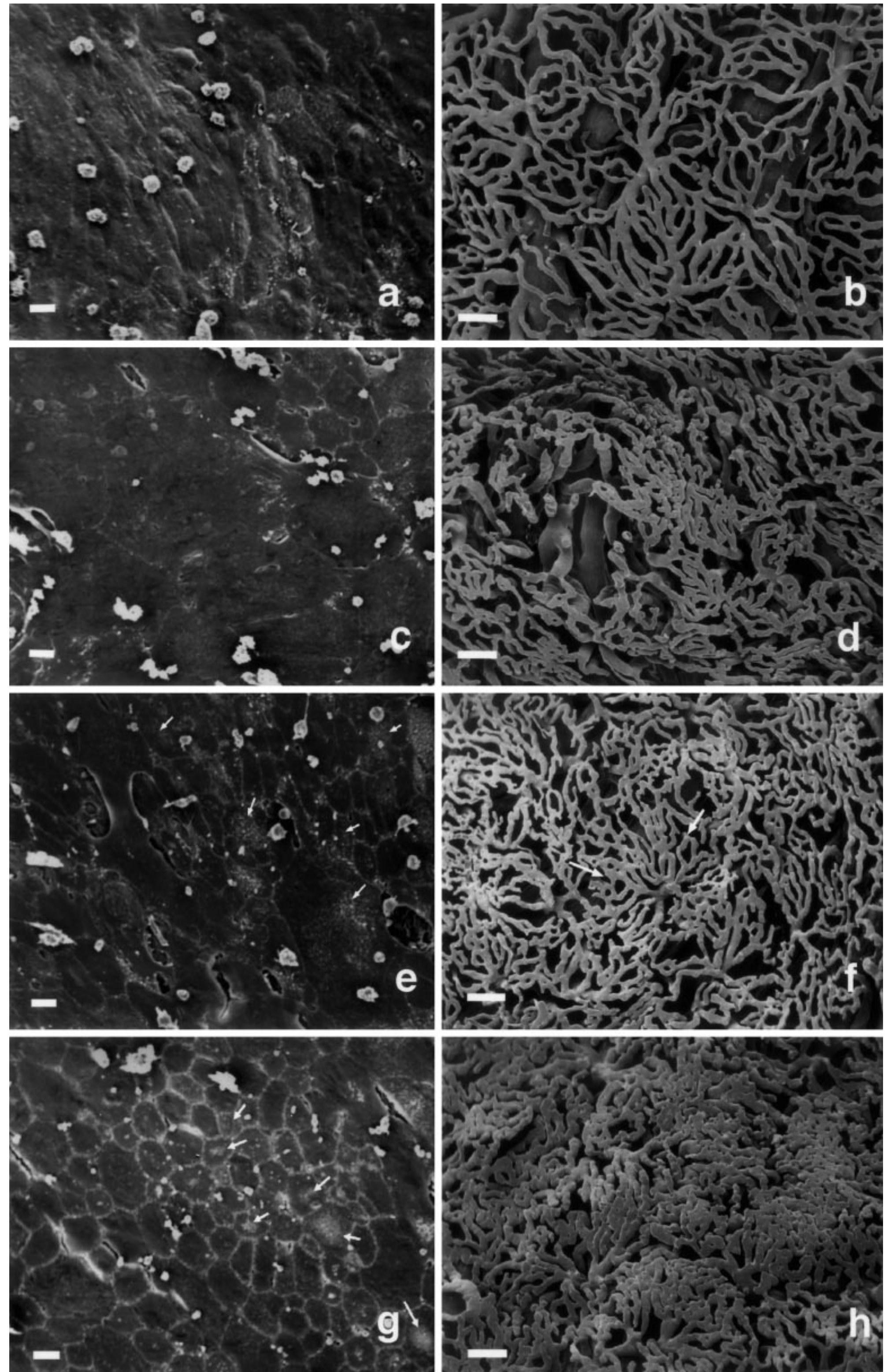

Fig. 4a–h Photomicrographs of the RPE (**a, c, e, g**) and the corrosion vascular casts (**b, d, f, h**) at the center of the debrided area at 7 and 14 days and 4 and 8 weeks after the surgery by scanning electron microscopy. **a, b** photomicrographs of the RPE and the vascular cast 7 days after surgery, respectively. **c, d** Photomicrographs of the RPE and the vascular cast 14 days after surgery. **e, f** Photomicrographs of the RPE and the vascular cast 4 weeks after surgery. *Arrows* Apical microvilli of the RPE (**e**); lobular structure of the choriocapillaris (**f**). **g. h** Photomicrographs of the RPE and the vascular cast 8 weeeks after surgery. *Arrows* Apical microvilli of the RPE. *Bar* 50 μm

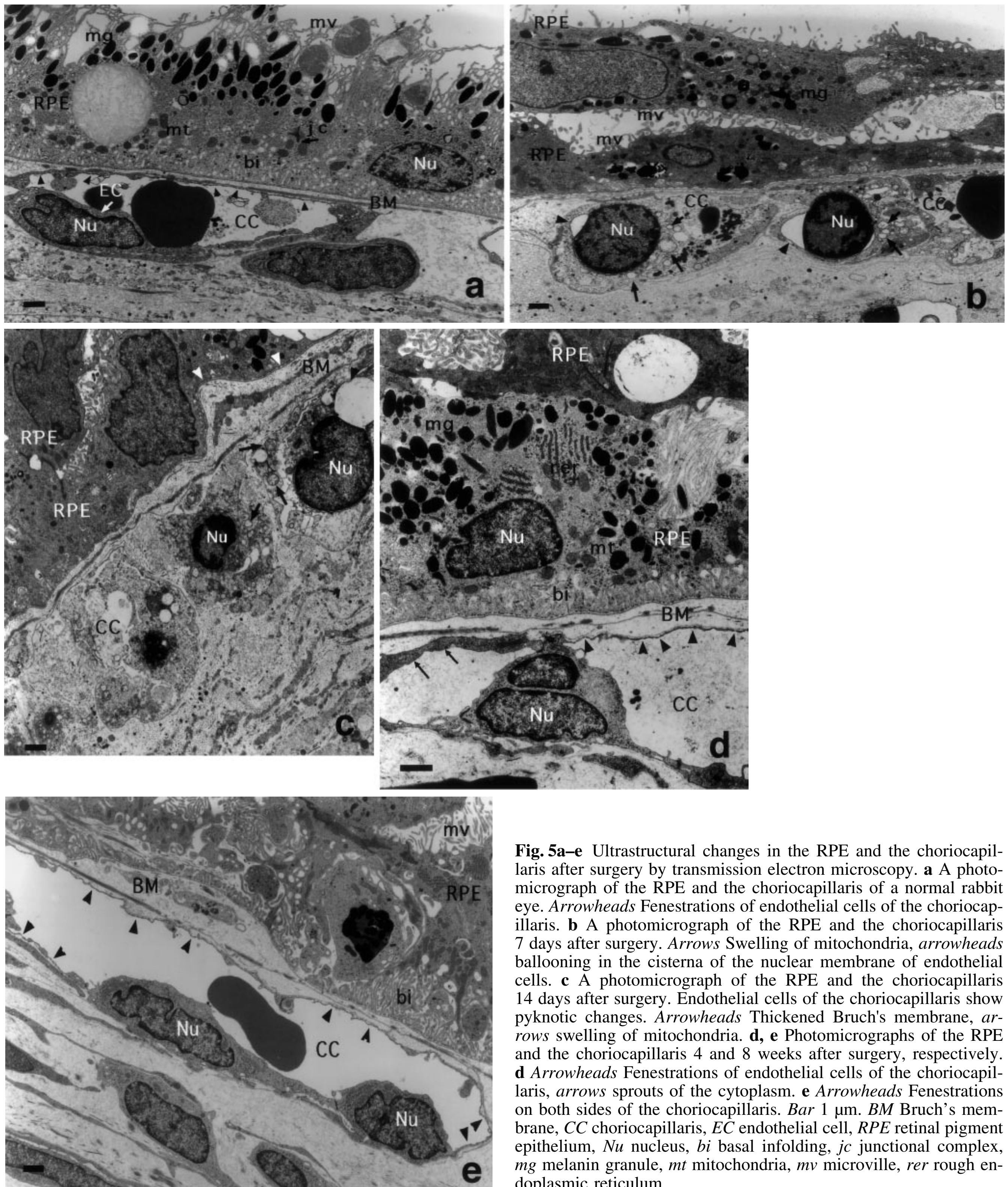

Fig. 5a–e Ultrastructural changes in the RPE and the choriocapillaris after surgery by transmission electron microscopy. **a** A photomicrograph of the RPE and the choriocapillaris of a normal rabbit eye. *Arrowheads* Fenestrations of endothelial cells of the choriocapillaris. **b** A photomicrograph of the RPE and the choriocapillaris 7 days after surgery. *Arrows* Swelling of mitochondria, *arrowheads* ballooning in the cisterna of the nuclear membrane of endothelial cells. **c** A photomicrograph of the RPE and the choriocapillaris 14 days after surgery. Endothelial cells of the choriocapillaris show pyknotic changes. *Arrowheads* Thickened Bruch's membrane, *arrows* swelling of mitochondria. **d, e** Photomicrographs of the RPE and the choriocapillaris 4 and 8 weeks after surgery, respectively. **d** *Arrowheads* Fenestrations of endothelial cells of the choriocapillaris, *arrows* sprouts of the cytoplasm. **e** *Arrowheads* Fenestrations on both sides of the choriocapillaris. *Bar* 1 µm. *BM* Bruch's membrane, *CC* choriocapillaris, *EC* endothelial cell, *RPE* retinal pigment epithelium, *Nu* nucleus, *bi* basal infolding, *jc* junctional complex, *mg* melanin granule, *mt* mitochondria, *mv* microville, *rer* rough endoplasmic reticulum

changes such as condensed chromatin in the nucleus, ballooning in the cisterna of the nuclear membrane (arrowheads), and swelling of mitochondria and other cytoplasmic organisms (arrows). Those endothelial cells also lost fenestrations; however, the basement membrane of the choriocapillaris appeared unaffected. Bruch's membrane showed a similar thickness to that in normal eyes. As shown in Fig. 5c, the RPE was still dedifferentiated with reduced basal infolding at 14 days after surgery. Bruch's membrane was focally thickened with excessive collagen bands and thick elastic layer (arrowheads in Fig. 5c). The lumen of the choriocapillaris was still obliterated with debris of pyknotic endothelial cells with swelling of mitochondria similar to those seen at 7 days after the surgery (see Fig. 5b). As shown in Fig. 5d, the RPE showed apparent basal infoldings, many melanin granules and mitochondria, and clear rough endoplasmic reticulum at 4 weeks after the surgery. However, the RPE cells were multi-layered and still lacking most of the apical microvilli. The lumen of the choriocapillaris was opened and fenestrations of the endothelial cells appeared (arrowheads). Sprouts of the cytoplasm were also observed in the endothelial cells of the choriocapillaris (arrows). Bruch's membrane was still thickened with excessive collagen and elastin bands. As shown in Fig. 5e, the RPE were still dedifferentiated with focally thickened Bruch's membrane at 8 weeks after surgery; however, the choriocapillaris was open and the cytoplasm and the nuclei of endothelial cells appeared normal. Fenestrations of the endothelial cells were observed not only on the side of Bruch's membrane but also on the choroidal side (arrowheads).

Discussion

This model of degeneration and regeneration of the choriocapillaris in rabbit was created by debridement of RPE in a large area (about 7 disk diameters) with removal of the overlying retina. Regeneration of the choriocapillaris was observed after 4 weeks with recovery of morphologic characteristics of the RPE in the debrided area. Several experimental models to induce degeneration of choriocapillaris have been examined to date. (1) Administration of sodium iodate caused selective destruction of RPE and secondary degeneration of the choriocapillaris [19]. (2) Surgical debridement of RPE with chemicals also caused degeneration of the choriocapillaris beneath the debrided area in pig [6]. (3) A rat strain with a hereditary retinal degeneration (Royal College of Surgeons rat) showed degeneration of the choriocapillaris adjacent to the degenerated RPE [4, 26]. Since the first model, involving administration of sodium iodate, showed patchy degeneration of the RPE throughout the fundus [17, 20], and since the third model, hereditary retinal degeneration, featured inability of phagocytosis by RPE [15], these models seemed more complicated than desirable to investigate mechanisms of regulation of choriocapillaris by RPE. Therefore, we developed a rabbit model in which degeneration of choriocapillaris can be easily created and surgically controlled. Our model included regeneration of the choriocapillaris because no pharmacologic reagent was used to inhibit the healing of RPE in the debrided area.

Although the debrided area was large (about 7 disk diameters), RPE from the debrided edge proliferated and migrated to cover the denuded Bruch's membrane in 7 days after surgery. Fluorescein angiography showed that tight junctions among the replaced RPE in the debrided area were not fully developed until 14 days after surgery. Our results corresponded to a previous study in that the RPE at the wound edge resurfaced the debrided area in 7 days after surgical debridement [14]. Similar responses by the surviving RPE around the degenerated RPE were observed in the animal models created by administration of sodium iodate or laser photocoagulation [17, 20, 31].

Previous studies have examined effects of surgical debridement of RPE on degeneration of choriocapillaris in vivo [5, 6, 14, 24, 37]. A previous study which created a large debridement of RPE showed degeneration of choriocapillaris [14]; however, our results did not correspond to some studies which showed that just mechanical debridement of RPE did not cause degeneration of the choriocapillaris [5, 6, 24, 37]. This discrepancy might be due to differences in sizes of the RPE debridement or simultaneous removal of surviving factors in the basement membrane of the RPE [5, 24, 37].

Although a previous study indicated that endothelial cells of the choriocapillaris showed apoptotic changes after administration of sodium iodate [21], in this study endothelial cells of the choriocapillaris showed necrotic changes in their nucleus and cytoplasm, such as condensed chromatin and swelling of mitochondria, by transmission electron microscopy. However, many of our observations regarding morphologic changes in the endothelial cells of the choriocapillaris beneath the debrided RPE correspond to previous published studies [17–19]. In the sodium iodate model, the endothelial cells of the choriocapillaris beneath the degenerated RPE were thickened and lost fenestrations as early as 1 day after administration of sodium iodate [19]. Then the choroidal endothelial cells died, leaving behind their basement membrane, which served as a guide or substrate for endothelial movement [17]. Regeneration of the choriocapillaris was shown to occur from choroidal venules, remnant choriocapillaris, and newly produced cells. Matured endothelial cells were observed 11 weeks after administration of sodium iodate [17, 18]. Our model demonstrated similar changes in endothelial cells of the choriocapillaris. At 7 days after surgery they showed necrosis, leaving their basement membrane behind. At 4 weeks after surgery the regenerated endothelial cells were observed with thick

sprouts of the cytoplasm. The choriocapillaris was recanalized after 4 weeks. Further studies are needed to clarify the cytopathic effects of RPE debridement on the endothelial cells of the choriocapillaris.

In our model the degenerated choriocapillaris apparently began to regenerate between 4 and 8 weeks after the surgery. These results corresponded to the timing of the recovery of morphological characteristics in the regenerated RPE such as apical microvilli and redistribution of melanin granules and mitochondria in the cytoplasm. Our observations corresponded to previous studies that observed thickening of Bruch's membrane and pleomorphic RPE during the regeneration processes [14, 21, 24, 29]. Thickening of Bruch's membrane appeared to be caused by overproduction of extracellular matrix by regenerating RPE and fibroblasts which invaded Bruch's membrane from the choroid. Endothelial cells of the choriocapillaris recovered their morphological characteristics at 4 and 8 weeks after the surgery; however, they did not show full recovery, since the fenestrations were observed on both of the choroidal side and the side of Bruch's membrane at 8 weeks. Since the overlying retina has been shown to influence the recovery of morphologic characteristics of the RPE after debridement [29], the absence of the overlying retina might influence the recovery of fenestrations of the choriocapillaris. Our results and those of others suggest that functionally recovered RPE may be necessary to maintain the underlying choriocapillaris [20, 21].

Previous studies proposed that factors from RPE (vascular modulating factors) might nourish and maintain choriocapillaris from clinical and experimental observations [6, 13, 19, 34, 39]. Recent studies have shown that basic fibroblast growth factor, vascular endothelial growth factor, endothelin-1, and insoluble molecules in the extracellular matrix produced by RPE act as survival factors for choroidal endothelial cells and vascular endothelial cells [1, 2, 16, 23, 33, 35, 38]. In this study we have not identified any factors from RPE; however, our system provides a convenient model to analyze survival factors for choriocapillaris in vivo.

In conclusion, we have developed a rabbit model of surgically induced degeneration and regeneration of the choriocapillaris by mechanical debridement of RPE. This model has four advantages compared to previously reported models: (1) The debridement of RPE in a large area was achieved because the RPE was exposed and observed directly under an operating microscope. (2) The RPE cells were debrided mechanically without chemicals to avoid any pharmacologic effects. (3) The debrided RPE cells were able to be removed from the vitreous cavity, preventing the debrided RPE from reattachment onto the bared Bruch's membrane. This made it possible to evaluate effects of the replaced RPE on the choriocapillaris. (4) The surgery is straightforward and highly reproducible, since the rabbit retina is avascular. No hemorrhage occurred when the detached retina was removed. A disadvantage of our model was the absence of the retina overlying the debrided area, which influenced recovery of the RPE [29]. However, the absence of the overlying retina has not been shown to cause any degeneration of the choriocapillaris.

This model may provide a useful tool to explore treatments for choroidal neovascular diseases such as age-related macular degeneration. Further studies are needed to identify potential factors from RPE which act as survival factors for choriocapillaris in vivo.

Acknowledgements The authors wish to thank Ms. Rhonda Grebe for excellent help in this study. Grant support was provided by the Core Grant of the Johns Hopkins University (P01-EY01765) and the Man Power Award of Research to Prevent Blindness, Inc.

References

1. Alon T, Hemo I, Itin A, Pe'er J, Stone J, Keshet E (1995) Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. Nat Med 1: 1024–1028
2. Benjamin LE, Keshet E (1997) Conditional switching of vascular endothelial growth factor (VEGF) expression in tumors: induction of endothelial cell shedding and regression of hemangioblastoma-like vessels by VEGF withdrawal. Proc Natl Acad Sci USA 94: 8761–8766
3. Burns MS, Hartz MJ (1992) The retinal pigment epithelium induces fenestration of endothelial cells in vivo. Curr Eye Res 9: 863–873
4. Caldwell RB (1989) Extracellular matrix alterations precede vascularization of the retinal pigment epithelium in dystrophic rats. Curr Eye Res 8: 907–921
5. Del Priore LV, Hornbeck R, Kaplan HJ, et al (1995) Debridement of the pig retinal pigment epithelium in vivo. Arch Ophthalmol 113: 939–944
6. Del Priore LV, Kaplan HJ, Hornbeck R, Jones Z, Swinn M (1996) Retinal pigment epithelial debridement as a model for the pathogenesis and treatment of macular degeneration. Am J Ophthalmol 122: 629–643
7. Desai VN, Del Priore LV, Kaplan HJ (1995) Choriocapillaris atrophy after submacular surgery in the presumed ocular histoplasmosis syndrome. Arch Ophthalmol 113: 409–410
8. Ernest JT (1994) Choroidal circulation. In: Ryan S, Ogden TE (eds) Retina. Mosby, St. Louis, pp76–80
9. Friedman E, Smith TR, Kuwabara T (1963) Senile choroidal vascular patterns and drusen. Arch Ophthalmol 69: 220–230

10. Fukushima I, Kusaka K, Takahashi K, et al (1995) Choroidal neovascularization with indocyanine green infrared fluorescence angiography and vascular cast preparation. Nippon Ganka Gakkai Zasshi 99: 47–58
11. Gartner S, Henkind P (1982) Pathology of retinitis pigmentosa. Ophthalmology 89: 1425–1432
12. Green WR, Key SN III (1977) Senile macular degeneration: a histopathologic study. Trans Am Ophthalmol Soc 75: 180–254
13. Henkind P, Gartner S (1983) The relationship between retinal pigment epithelium and the choriocapillaris. Trans Ophthalmol Soc UK 103: 444–447
14. Heriot WJ, Machemer R (1992) Pigment epithelial repair. Graefe's Arch Clin Exp Ophthalmol 230: 91–100
15. Herron WL, Riegel BW, Myers OE, Rubin ML (1969) Retinal dystrophy in the rat – a pigment epithelial disease. Invest Ophthalmol Vis Sci 8: 595–604
16. Karsan A, Yee E, Poirier GG, Zhou P, Craig R, Harlan JM (1997) Fibroblast growth factor-2 inhibits endothelial cell apoptosis by Bcl-2-dependent and independent mechanisms. Am J Pathol 151: 1775–1784
17. Korte GE (1989) Choriocapillaris regeneration in the rabbit. Ultrastructure of new endothelial tube formation. Invest Ophthalmol Vis Sci 30: 1938–1950
18. Korte GE, Pua F (1988) Choriocapillaris regeneration in the rabbit. A study with vascular casts. Acta Anat 133: 224–228 epithelium. Int Rev Cytology 152: 223–263
19. Korte GE, Reppucci V, Henkind P (1984) RPE destruction causes choriocapillaris atrophy. Invest Ophthalmol Vis Sci 25: 1135–1145
20. Korte GE, Burns MS, Bellhorn RW (1989) Epithelium-capillary interactions in the eye: The retinal pigment epithelium and the choriocapillaris. Int Rev Cytol 114: 221–248.
21. Korte GE, Perlman JI, Pollack A (1994) Regeneration of mammalian retinal pigment epithelium. Int. Rev. Cytol 152: 223–263
22. Leonard DS, Zhang X-G, Panozzo G, Sugino IK, Zarbin MA (1997) Clinicopathologic correlation of localized retinal pigment epithelium debridement. Invest Ophthalmol Vis Sci. 38: 1094–1109
23. Liu X, Ye X, Yanoff M, Li W (1997) Extracellular matrix of retinal pigment epithelium regulates choriocapillaris endothelial survival in vitro. Exp Eye Res 65: 117–126
24. Lopez PF, Yan Q, Kohen L, et al (1995) Retinal pigment epithelial wound healing in vivo. Arch Ophthalmol 113: 1437–1446
25. Mancini MA, Frank RN, Keirn RJ, Kennedy A, Khoury JK (1986) Does the retinal pigment epithelium polarize the choriocapillaris? Invest Ophthalmol Vis Sci 27: 336–345
26. May CA, Horneber M, Lutjen-Drecoll E (1996) Quantitative and morphological changes of the choroid vasculature in RCS rats and congenic controls. Exp Eye Res 63: 75–84
27. Nasir MA, Sugino I, Zarbin MA (1997) Decreased choriocapillaris perfusion following surgical excision of choroidal neovascular membranes in age-related macular degeneration. Br J Ophthalmol 81: 481–489
28. Ormerod LD, Puklin JE, Frank RN (1994) Long-term outcomes after the surgical removal of advanced subfoveal neovascular membranes in age-related macular degeneration. Ophthalmology 101: 1201–1210
29. Ozaki S, Kita M, Yamana T, Negi A, Honda Y (1997) Influence of the sensory retina on healing of the rabbit retinal pigment epithelium. Curr Eye Res 16: 349–358
30. Pollack JS, Del Priore LV, Smith ME, Feiner MA, Kaplan HJ (1996) Postoperative abnormalities of the choriocapillaris in exudative age-related macular degeneration. Br J Ophthalmol 80: 314–318
31. Pollack A, Korte GE (1990) Repair of retinal pigment epithelium and its relationship with capillary endothelium after krypton laser photocoagulation. Invest Ophthalmol Vis Sci 31: 890–898
32. Pollack A, Korte GE (1992) Vascular remodeling after laser photocoagulation. Concurrent regeneration and atrophy. Acta Anat 143: 151–159
33. Roberts WG, Palade GE (1995) Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. J Cell Sci 108: 2369–2379
34. Sarks SH, Sarks JP (1994) Age-related macular degeneration-atrophic form. In: Ryan S, Ogden TE (eds) Retina. Mosby, St. Louis, pp 1071–1102
35. Shichiri M, Kato H, Marumo F, Hirata Y (1997) Endothelin-1 as an autocrine/ paracrine apoptosis survival factor for endothelial cells. Hypertension 30: 1198–1203
36. 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
37. Valentino TL, Kaplan HJ, Del Priore LV, Fang S-R, Berger A, Silverman MS (1995) Retinal pigment epithelial repopulation in monkeys after submacular surgery. Arch Ophthalmol 113: 932–938
38. Watanabe Y, Dvorak HF (1997) Vascular permeability factor/ vascular endothelial growth factor inhibits anchorage-disruption-induced apoptosis in microvessel endothelial cells by inducing scaffold formation. Exp Cell Res 233: 340–349
39. Young RW (1987) Pathophysiology of age-related macular degeneration. Surv Ophthalmol 31: 291–306