

Ultrastructure of Surgically Excised Subfoveal Neovascular Membranes

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We studied the ultrastructural features of four consecutive subfoveal neovascular membranes(SFNM) associated with age-related macular degeneration. Cellular components of the membranes included retinal pigment epithelial(RPE) cells, endothelium-lined vascular channels, macrophages, myofibroblasts, fibrocytes, glial cells, erythrocytes, and lymphocytes. Extracellular interstitial constituents included collagen fibrils, basal laminar deposits, fibrin and young elastic fibrils. These findings show that SFNMs consist of various cells originating from surrounding tissues and vessels. Among these RPE cells and macrophages are the main cellular components and in conjunction with various extracellular matrix, especially collagen, may play an important role in the formation and maintenance of the membranes.

Key words: age-related macular degeneration, subfoveal neovascular membrane, ultrastructural feature

INTRODUCTION

Subretinal neovascularization(SRN) is an acquired abnormality resulting from a complicated pathologic response to a wide variety of disease processes that affect the retinal pigment epithelium (RPE)-Bruch's membrane-choriocapillaris complex. SRN is the determinant of the disciform process, which is responsible for more than 80% of cases of significant visual loss in patients with age-related macular degeneration(ARMD).¹ The evolution of the disciform process has been seen to involve repeated episodes of serous and hemorrhagic detachments due to leakage from SRN, with the formation of a subretinal fibrovascular membrane

and eventual cicatrization.¹⁻³ The exact pathogenesis of SRN has not been established, however. With recent advances in vitreoretinal microsurgical instrumentation and techniques, a direct surgical approach for the excision of subfoveal neovascular membrane(SFNM) is now possible,^{4,5} and the histopathologic features of such membranes have been studied.⁶⁻⁹

Recently, we surgically excised four consecutive SFNMs from patients with age-related macular degeneration and examined the ultrastructural features of the specimens to understand the pathogenesis and clinicopathologic features of the membranes.

MATERIALS AND METHODS

Surgical removal of symptomatic SFNMs associated with age-related macular degeneration was performed in four consecutive patients in whom laser treatment seemed unlikely to be useful in

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preserving central vision.

The surgical technique used was similar to that reported for removal of SFNMs in ARMD and presumed ocular histoplasmosis.^{4,5} Briefly, the surgical procedures included pars plana vitrectomy, retinotomy, separation of the membrane from the surrounding tissue, removal of the membrane, and air-fluid exchange.

Excised SFNMs were promptly fixed in 2.5% glutaraldehyde for two hours and postfixated with 0.1 M cacodylate buffer and 1% osmium tetroxide. After standard dehydration, the specimens were embedded in epoxy resin. Semithin sections (1.0 μm) were cut and stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate for transmission electron microscopy. Previously reported criteria were used for ultrastructural identification of cellular and extracellular constituents of the membranes.¹⁰

RESULTS

We analyzed the pathologic findings of four surgically-excised SFNMs characterized by fibrovascular tissue subjacent to the RPE cells. The vascular channels were present in all cases and were composed of endothelium-lined capillaries surrounded by pericytes (Fig. 1). All specimens contained RPE cells along the margin or within the

membranes; these cells showed numerous apical microvilli and tight intercellular junctions, and were surrounded by irregular basement membrane (Fig. 2). Myofibroblasts and fibrocytes were present in four and two membranes, respectively (Figs. 3 & 4). Glial cells were detected in three specimens and contained abundant intermediate filaments within their cytoplasm. Chronic inflammatory cells were commonly observed in the membranes. Macrophages and lymphocytes were detected in all four specimens and plasma cells were found in one membrane (Figs. 1 & 4). Erythrocytes and ghost

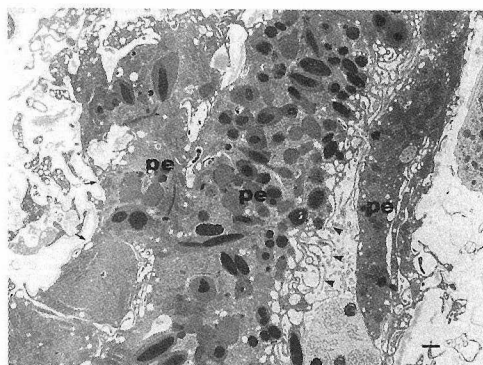


Fig. 2. RPE cells (pe) was seen within the membranes. They showed numerous apical microvilli (arrow heads) and were surrounded by irregular basal lamina (arrows). Bar = 1 μm

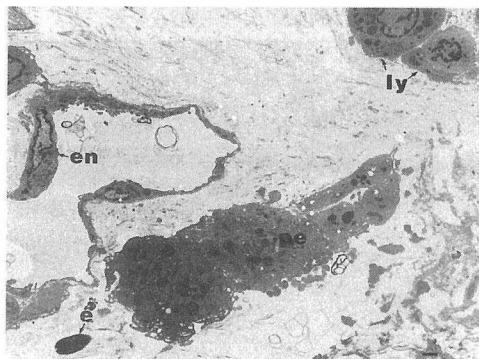


Fig. 1. A vascular channel is seen primarily composed of thin endothelial cells (en) surrounded by scanty basement membrane. Scattered lymphocytes (ly), an RPE cell (pe), and an erythrocyte (e) were also seen in the abundant collagen stroma. Bar = 1 μm



Fig. 3. Electron micrograph showing a myofibroblast. This cell contained numerous intracytoplasmic organelles and the fusiform aggregations of fine filaments (arrow heads) beneath the plasma membrane. Bar = 1 μm

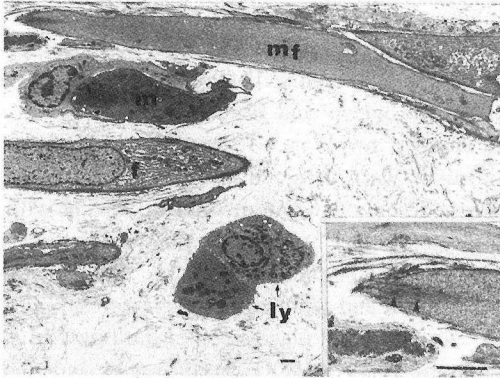


Fig. 4. Electron micrograph showing various cellular elements in a membrane. A fibrocyte (f), a myofibroblast-like cell (mf), a macrophage (m) and lymphocytes (ly) were seen in abundant collagen stroma. Inset: magnified view showing aggregations of fine filaments (arrow head) within the myofibroblast. Bar = 1 μ m

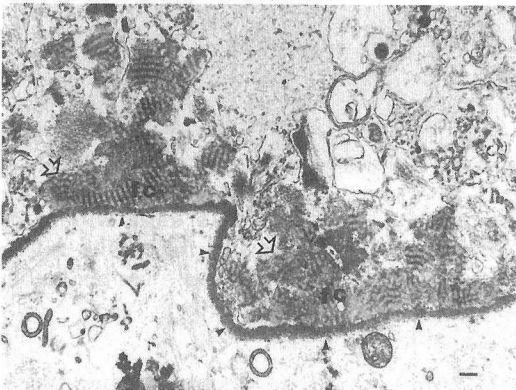


Fig. 5. Electron micrograph showing BLD (open arrows) containing long-spaced collagen (fc) and located along the inner portion of Bruch's membrane (arrow heads). Bar = 1 μ m

erythrocytes were each found in three specimens and showed evidence of previous hemorrhage within the membranes (Figs. 1, 7, & 8). As an extracellular component, 100nm-collagen was found in all specimens (Figs. 1 & 4). Young elastin-fibrils were detected in one membrane. Basal laminar deposits (BLD), located between the RPE and the inner collagenous layer of Bruch's membrane, were detected in two membranes and included the long-spaced collagen and homogenous granular material

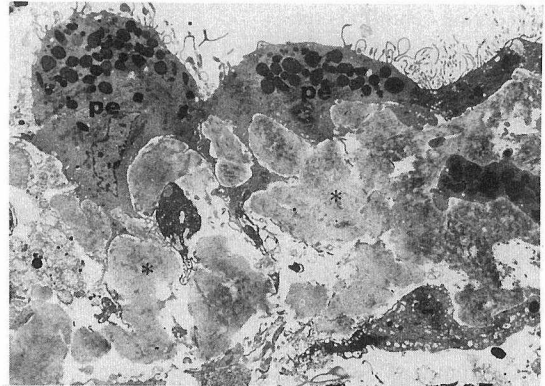


Fig. 6. Abnormal amorphous basement membrane material (asterisks) was seen beneath RPE (pe) was seen. Bar = 1 μ m

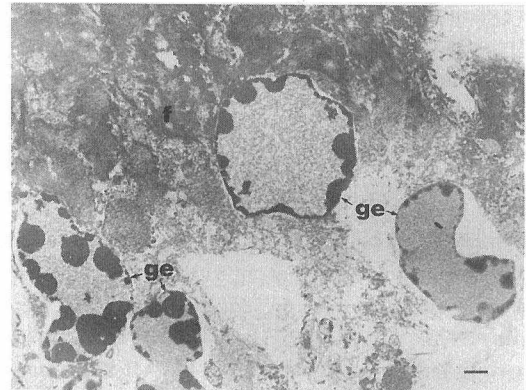


Fig. 7. Electron micrograph showing ghost erythrocytes (ge) and abundant fibrin (f). Bar = 1 μ m

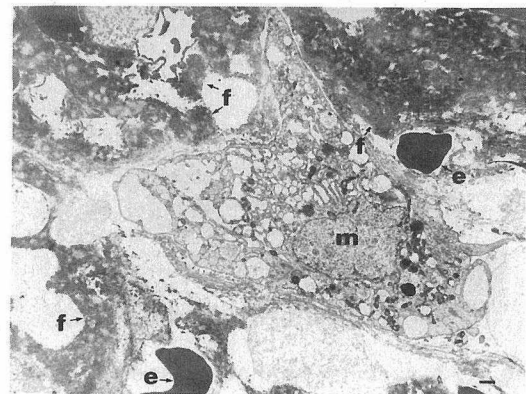


Fig. 8. A lipidized cell (m) that may present a macrophage or RPE cell was present in a membrane. Erythrocytes (e) and fibrin (f) were also seen. Bar = 1 μ m

(Figs. 5 & 6). Fragments of Bruch's membranes were observed in two specimens (Fig. 5). Fibrin was detected in all four membranes (Figs. 7 & 8) and a lipidized cell that may have represented a macrophage or RPE was present in one (Fig. 8).

DISCUSSION

The histopathologic features of SFNM have been studied in autopsy eyes,^{3,11} experimental animal models,^{12,13} and surgically excised specimens.⁶⁻⁹ Some of these findings support the concept that SFNM, regardless of etiology, represents a nonspecific healing response to a pathologic stimulus occurring in the RPE-Bruch's membrane-choriocapillaris complex. Gehrs et al.⁹ hypothesized that choroidal neovascularization gained access to the sub-RPE space by eroding Bruch's membrane and passing to the zone beneath the RPE basement membrane. Kalebic et al.¹⁴ demonstrated the ability of endothelial cells to degrade collagen when stimulated by retinal extracts and Heriot et al.¹⁵ showed that endothelial cells may create their own defect in Bruch's membrane rather than growing through an existing break.

We examined the ultrastructural features of four surgically excised SFNMs from patients with ARMD and our findings were similar to those of other authors.^{6-9,11} The excised SFNMs in our study were characterized by fibrovascular tissues subjacent to the RPE. Cellular components of the membranes include RPE cells, endothelium-lined vascular channels, macrophages, lymphocytes, myofibroblasts, fibrocytes, glial cells, erythrocytes, ghost erythrocytes, and plasma cells. Of these, RPE and macrophages were most prominent in the membranes. The RPE cells affected would proliferate and undergo metaplasia and contribute to the organizing scar; together with the fibrovascular tissue invading from the choroid, they also appears to represent a homeostatic reparative process required to reestablish the blood-ocular barrier.¹⁶ Chronic inflammatory cells, including macrophages and lymphocytes, were found in all our cases. The inflammatory cells may have been introduced via endothelium-lined vascular channels within the membranes or may have reached into the membranes from the adjacent retina or choroid. The

inflammatory component of the membranes appears to be involved as a dynamic component in membrane formation, including the release of enzymatic products and remodeling.^{6,17,18} Penfold et al.¹⁹ demonstrated that chronic inflammatory cells actually engulf material from Bruch's membrane. The precise role that these cells may play in the pathogenesis of SRN is not understood; it is probable, however, that they play an important role in the formation and maintenance of the membranes.^{6,8,18} Ultrastructural evidence of macrophages was reported in 80% of SFNMs in ARMD.⁶ The vascular channels were present in all our cases and were composed of endothelium-lined capillaries surrounded by pericytes. The vascular endothelium is considered to be of choroidal origin and enters the subretinal space via a break in Bruch's membrane,²⁰ though a histopathologically detectable defect in this membrane is not necessary for SRN.²¹ Myofibroblasts were present in all membrane. Although their origin is not clear, these cells may develop after transformation of fibrocytes²² or RPE and appear to be associated with the contraction of SFNM and the formation of RPE tears.² Scar contraction within the membrane may lead to the formation of new breaks in Bruch's membrane, as proposed by Lopez and Green.¹⁸ Fibrocytes were demonstrated in two of our cases; these cells are probably of choroidal origin, although they may also have originated from transformed macrophages.²² Fibrocytes, perhaps introduced through a defect in Bruch's membrane, may have produced new collagen. Glial cells were found in three membranes in our study and appeared to have contributed to these membranes through the neurosensory retina.²³

Extracellular components of the our specimens included collagen fibrils, fibrin, abnormal basement membrane material, basal laminar deposit, fragments of Bruch's membrane, and elastin. Of these, collagen and fibrin were found in all membranes. Collagen stromas consisted of 100 nm collagen fibrils and were thought to have originated from fibrocytes within the membranes. BLD was detected in two membranes in our study. Diffuse drusen within Bruch's membrane is considered to be a precursor of ARMD.^{3,6,24,25} Progressive diffuse accumulation of BLD on the basal side of the RPE

within the macular area has been associated with progressive degeneration of the RPE and probably predisposes this and its basement membrane to separation from the remainder of Bruch's membrane.² This diffuse injury may result in a reparative choroidal fibrovascular ingrowth (SRN) that in itself may lead to further pathologic changes such as subretinal hemorrhage, causing acute worsening of vision.^{3,18} Ultrastructurally, SRN primarily consists of aggregates of wide-spaced collagen with a characteristic banded pattern of approximately 120 nm.²⁴ Grossniklaus et al.²⁵ suggest that the presence of BLD in surgically excised SFNM is highly suggestive of ARMD. Fragments of Bruch's membrane were detected in two membranes of our cases and were thought to be derived from surgical delamination of Bruch's membrane. Lopes et al.⁶ demonstrated such fragments in 30% of their cases. Fibrin was observed in all our cases; its deposition may have served as a scaffold for the proliferation of fibrocellular tissue that formed the stroma of the membrane.⁶⁻⁸ The fibrin may be within the membrane or in the subneurosensory space at its periphery.²⁶

In selected cases, surgical removal of the membrane can be accomplished without the removal of underlying RPE and choroidal structures. Minimal retinal damage occurs and visual results are relatively good.

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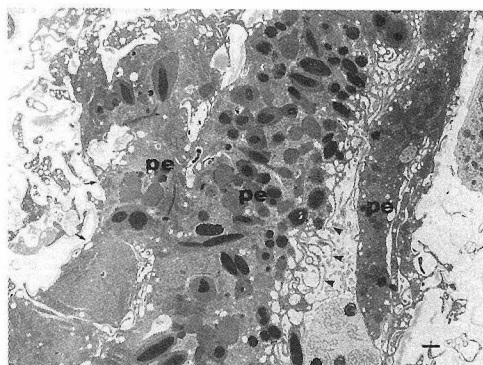


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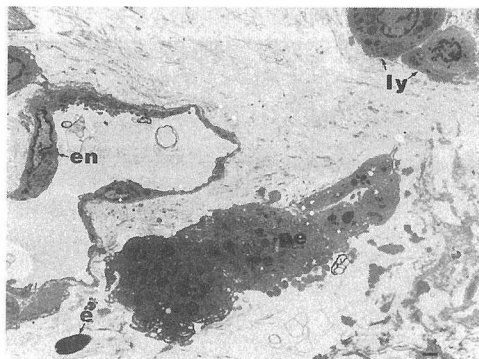


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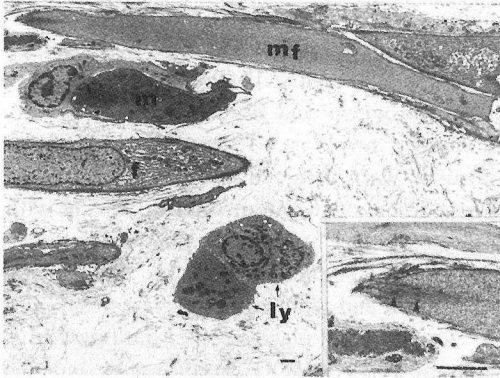


Fig. 4. Electron micrograph showing various cellular elements in a membrane. A fibrocyte (f), a myofibroblast-like cell (mf), a macrophage (m) and lymphocytes (ly) were seen in abundant collagen stroma. Inset: magnified view showing aggregations of fine filaments (arrow head) within the myofibroblast. Bar = 1 μ m

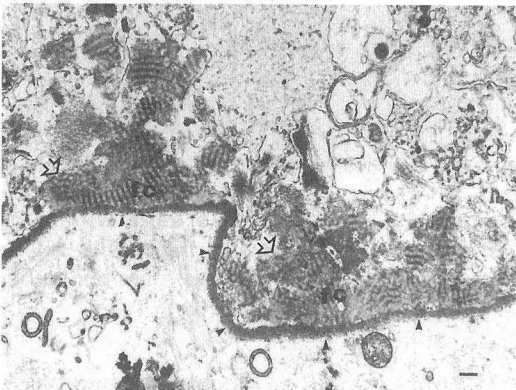


Fig. 5. Electron micrograph showing BLD (open arrows) containing long-spaced collagen (fc) and located along the inner portion of Bruch's membrane (arrow heads). Bar = 1 μ m

erythrocytes were each found in three specimens and showed evidence of previous hemorrhage within the membranes (Figs. 1, 7, & 8). As an extracellular component, 100nm-collagen was found in all specimens (Figs. 1 & 4). Young elastin-fibrils were detected in one membrane. Basal laminar deposits (BLD), located between the RPE and the inner collagenous layer of Bruch's membrane, were detected in two membranes and included the long-spaced collagen and homogenous granular material

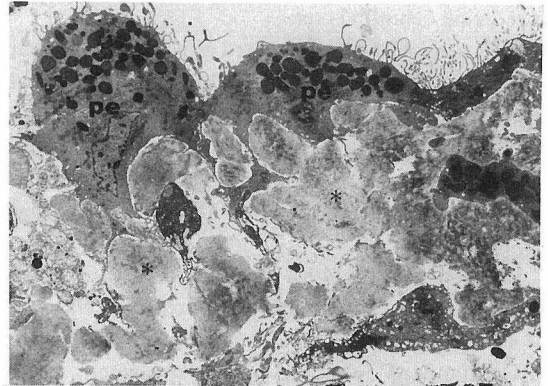


Fig. 6. Abnormal amorphous basement membrane material (asterisks) was seen beneath RPE (pe) was seen. Bar = 1 μ m

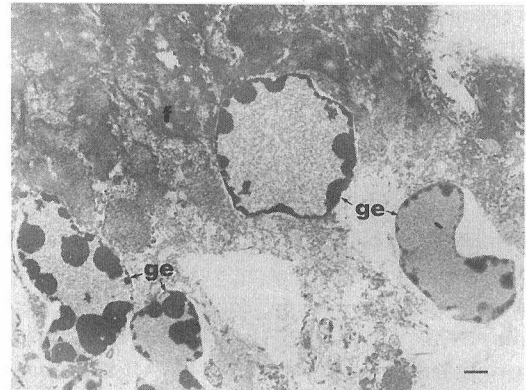


Fig. 7. Electron micrograph showing ghost erythrocytes (ge) and abundant fibrin (f). Bar = 1 μ m

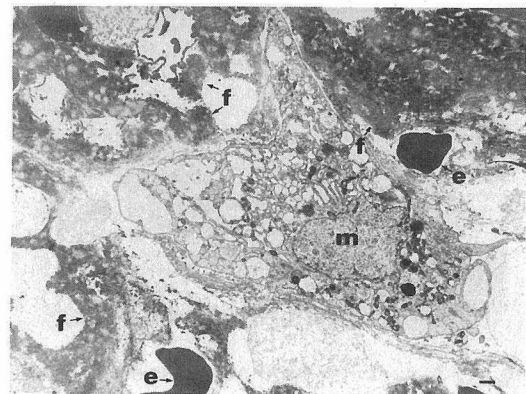


Fig. 8. A lipidized cell (m) that may present a macrophage or RPE cell was present in a membrane. Erythrocytes (e) and fibrin (f) were also seen. Bar = 1 μ m

(Figs. 5 & 6). Fragments of Bruch's membranes were observed in two specimens (Fig. 5). Fibrin was detected in all four membranes (Figs. 7 & 8) and a lipidized cell that may have represented a macrophage or RPE was present in one (Fig. 8).

DISCUSSION

The histopathologic features of SFNM have been studied in autopsy eyes,^{3,11} experimental animal models,^{12,13} and surgically excised specimens.⁶⁻⁹ Some of these findings support the concept that SFNM, regardless of etiology, represents a nonspecific healing response to a pathologic stimulus occurring in the RPE-Bruch's membrane-choriocapillaris complex. Gehrs et al.⁹ hypothesized that choroidal neovascularization gained access to the sub-RPE space by eroding Bruch's membrane and passing to the zone beneath the RPE basement membrane. Kalebic et al.¹⁴ demonstrated the ability of endothelial cells to degrade collagen when stimulated by retinal extracts and Heriot et al.¹⁵ showed that endothelial cells may create their own defect in Bruch's membrane rather than growing through an existing break.

We examined the ultrastructural features of four surgically excised SFNMs from patients with ARMD and our findings were similar to those of other authors.^{6-9,11} The excised SFNMs in our study were characterized by fibrovascular tissues subjacent to the RPE. Cellular components of the membranes include RPE cells, endothelium-lined vascular channels, macrophages, lymphocytes, myofibroblasts, fibrocytes, glial cells, erythrocytes, ghost erythrocytes, and plasma cells. Of these, RPE and macrophages were most prominent in the membranes. The RPE cells affected would proliferate and undergo metaplasia and contribute to the organizing scar; together with the fibrovascular tissue invading from the choroid, they also appears to represent a homeostatic reparative process required to reestablish the blood-ocular barrier.¹⁶ Chronic inflammatory cells, including macrophages and lymphocytes, were found in all our cases. The inflammatory cells may have been introduced via endothelium-lined vascular channels within the membranes or may have reached into the membranes from the adjacent retina or choroid. The

inflammatory component of the membranes appears to be involved as a dynamic component in membrane formation, including the release of enzymatic products and remodeling.^{6,17,18} Penfold et al.¹⁹ demonstrated that chronic inflammatory cells actually engulf material from Bruch's membrane. The precise role that these cells may play in the pathogenesis of SRN is not understood; it is probable, however, that they play an important role in the formation and maintenance of the membranes.^{6,8,18} Ultrastructural evidence of macrophages was reported in 80% of SFNMs in ARMD.⁶ The vascular channels were present in all our cases and were composed of endothelium-lined capillaries surrounded by pericytes. The vascular endothelium is considered to be of choroidal origin and enters the subretinal space via a break in Bruch's membrane,²⁰ though a histopathologically detectable defect in this membrane is not necessary for SRN.²¹ Myofibroblasts were present in all membrane. Although their origin is not clear, these cells may develop after transformation of fibrocytes²² or RPE and appear to be associated with the contraction of SFNM and the formation of RPE tears.² Scar contraction within the membrane may lead to the formation of new breaks in Bruch's membrane, as proposed by Lopez and Green.¹⁸ Fibrocytes were demonstrated in two of our cases; these cells are probably of choroidal origin, although they may also have originated from transformed macrophages.²² Fibrocytes, perhaps introduced through a defect in Bruch's membrane, may have produced new collagen. Glial cells were found in three membranes in our study and appeared to have contributed to these membranes through the neurosensory retina.²³

Extracellular components of the our specimens included collagen fibrils, fibrin, abnormal basement membrane material, basal laminar deposit, fragments of Bruch's membrane, and elastin. Of these, collagen and fibrin were found in all membranes. Collagen stromas consisted of 100 nm collagen fibrils and were thought to have originated from fibrocytes within the membranes. BLD was detected in two membranes in our study. Diffuse drusen within Bruch's membrane is considered to be a precursor of ARMD.^{3,6,24,25} Progressive diffuse accumulation of BLD on the basal side of the RPE

within the macular area has been associated with progressive degeneration of the RPE and probably predisposes this and its basement membrane to separation from the remainder of Bruch's membrane.² This diffuse injury may result in a reparative choroidal fibrovascular ingrowth (SRN) that in itself may lead to further pathologic changes such as subretinal hemorrhage, causing acute worsening of vision.^{3,18} Ultrastructurally, SRN primarily consists of aggregates of wide-spaced collagen with a characteristic banded pattern of approximately 120 nm.²⁴ Grossniklaus et al.²⁵ suggest that the presence of BLD in surgically excised SFNM is highly suggestive of ARMD. Fragments of Bruch's membrane were detected in two membranes of our cases and were thought to be derived from surgical delamination of Bruch's membrane. Lopes et al.⁶ demonstrated such fragments in 30% of their cases. Fibrin was observed in all our cases; its deposition may have served as a scaffold for the proliferation of fibrocellular tissue that formed the stroma of the membrane.⁶⁻⁸ The fibrin may be within the membrane or in the subneurosensory space at its periphery.²⁶

In selected cases, surgical removal of the membrane can be accomplished without the removal of underlying RPE and choroidal structures. Minimal retinal damage occurs and visual results are relatively good.

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