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Electron Microscopic Study on the Development of Precapsular Layer in Eyes With Exfoliation Syndrome

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Purpose: To search for a pathogenic mechanism for the formation of the precapsular layer on the anterior lens surface in pre-exfoliative eyes.

Methods: We examined anterior lens capsules obtained during surgery from 38 patients (control, 16; exfoliation suspect, 10; exfoliation, 12) by transmission electron microscopy.

Results: A precapsular layer was found in 5 of 16 controls and 7 of 10 exfoliation suspects. It was composed mainly of microfibrils 5–8 nm in diameter. Degenerated zonular fragments were occasionally found interspersed in, and sometimes merged with microfibrils of the precapsular layer.

Conclusion: Zonular fibers might contribute to the formation of the precapsular layer in pre-exfoliation stages. **Jpn J Ophthalmol 2000;44:9–14** © 2000 Japanese Ophthalmological Society

Key Words: Electron microscopy, exfoliation syndrome, microfibril, precapsular layer, zonular fibril.

Introduction

There are several reports describing the clinical signs of eyes with the exfoliation syndrome.[1–6] These signs include pigment deposition on the corneal endothelium, loss of pupillary ruff, pigment deposition on the iris sphincter region, iris transillumination defects, anterior chamber pigment dispersion after pupillary dilatation, and dense pigmentation of the trabecular meshwork. Even in the absence of biomicroscopically evident exfoliation material in the anterior segment, these pigment-related signs correlated with ultrastructural findings of exfoliation fibers in the conjunctiva of exfoliation-suspect patients in whom no typical exfoliation material was observed in the anterior ocular segments.[3] Another morphological study reported that a precapsular layer (PCL) on the anterior lens surface was frequently observed in specimens from exfoliation suspect patients with pigment-related ocular signs. This precapsular layer was composed of microfibrils, amorphous material, and granular inclusions, and may represent a precursor of typical exfoliation material.[7]

Several electron microscopic studies[8–10] showed that two mechanisms are involved in the distribution of the exfoliation material. In the anterior segment of the eye, the exfoliation material appears to originate from various cell types lining the anterior and posterior chambers, including iris pigment epithelium, ciliary nonpigmented epithelium, pre-equatorial lens epithelium, and corneal endothelium. Accumulation of exfoliation material on the central anterior lens capsule results from secondary deposition from the aqueous humor. Other authors reported that microfibrils in the tissues and zonular fibrils may develop into the exfoliation material.[11–15] The origin of the precapsular layer of pre-exfoliation and early exfoliation syndrome, however, remains unclear. We carried out this study to try to learn more about the development of the precapsular layer and its clinical correlation, and to search for a possible pathogenic mechanism for its formation.

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Materials and Methods

Thirty-eight anterior lens capsules were obtained during extracapsular cataract surgery in 38 patients, after having obtained informed consent from each patient. These patients had been previously examined by slit-lamp biomicroscopy and were divided into three groups: (1) clinically confirmed exfoliation, 12 patients, aged 62–94 years (mean = 74.25 years); (2) exfoliation suspects, 10 patients, aged 40–84 years (mean = 67.6 years); and (3) age-matched controls, 16 patients, aged 58–87 years (mean = 70.2 years). The diagnosis of exfoliation-suspect was made when there was no exfoliation material on the lens capsule and pupillary rim in either eye, but there were at least two of the following clinical signs as reported elsewhere:[3,6,7] poor mydriasis, iris stromal atrophy, pupillary ruff defect, pigment particles on the anterior lens surface, anterior chamber pigment dispersion after dilatation, and retrocorneal pigment deposition.

The anterior lens capsules were immersed immediately after anterior capsulotomy in a solution of 4% glutaraldehyde prepared in 0.1 M cacodylate buffer (pH 7.2) for 24 hours at 4°C. After postfixation in 1.0% buffered osmium tetroxide, the specimens were dehydrated in a graded series of alcohol and embedded in an epoxy resin (Epon 812). Ultrathin sections were cut on an ultramicrotome (Sorval, Porter-Blum MT2-B; Dupont Instruments, Newtown, CT, USA), stained with uranyl acetate-lead citrate and examined with a transmission electron microscope (JEM-100; CX Ltd, Tokyo). At least 3 ultrathin sections from different parts of each capsule were examined.

Results

Correlation of Clinical Signs and Electron Microscopic Findings

Exfoliation material was found ultrastructurally in 9 specimens from 12 patients with clinical exfoliation, but was not found in the specimens from the exfoliation suspects and the normal controls. A PCL of the lens was ultrastructurally identified on the anterior lens capsule in 7 of 10 exfoliation-suspect eyes and 5 of 16 control eyes. Neither exfoliation material nor a PCL was observed ultrastructurally in the specimens from 3 of the clinical exfoliation patients, from 3 of the clinical exfoliation suspects, or from 11 of the controls (Table 1).

Table 1. Correlation of Clinical Signs and Electron Microscopic Findings

	Electron Microscopic Finding		
	PEX mat	PCL	No PCL
Clinical exfoliation subjects (n = 12)	9	–	3
Suspected exfoliation subjects (n = 10)	–	7	3
Controls (n = 16)	–	5	11

PEX: exfoliation material, PCL: precapsular layer.

Ultrastructural Findings

Control subjects. In 5 of the 16 capsules obtained from control subjects, a PCL was found that was similar to that detected in exfoliation suspects, albeit thinner and patchy. The other capsules showed a relatively smooth surface with no evidence of microfibrils (Figure 1a).

Exfoliation suspects. On the surface of the anterior lens capsule, a PCL composed of a fine network of fibers that had the ultrastructural characteristics of elastic tissue microfibrils, amorphous material, and granular inclusions was observed in 7 of 10 exfoliation suspects. This PCL was 0.5–2.0 μm thick (Figure 1b). The microfibrils were variable in length, with a diameter of 5–8 nm, and showed banding with a periodicity of 12–14 nm. There was no clear demarcation between this layer and the capsule (Figure 2). In 3 cases, fragmented zonular bundles with the typical arrangement of 11–12 nm fibers showing a faint banding of 12–14 nm periodicity were observed interspersed with the PCL microfibrils in the periphery of the PCL (Figure 3A). In different areas of the PCL, much smaller fragments were found to be closely related to, and often passing imperceptibly into the PCL microfibrils (Figures 3B, 3C).

Clinical exfoliation subjects. In 9 of the 12 capsules obtained from clinically confirmed exfoliation eyes, aggregates of typical exfoliation material were observed in the form of randomly arranged tangles of electron-dense fibers, 30–50 nm in diameter and finer fibrils with electron-dense deposits embedded in an amorphous ground substance (Figure 1c). In areas free of exfoliation aggregates, a fibrillary area, composed of microfibrils 5–8 nm and 11–14 nm in diameter with a banding periodicity of 12–14 nm, and containing scattered larger exfoliation fibers was observed. In one sample, fragmented zonular bundles, similar to those described in the PCL, were observed interspersed between these microfibrils. Many of the microfibrils in these areas were observed to be fused together, while others showed deposition of some electron-dense material and subsequent thickening (Figure 4).

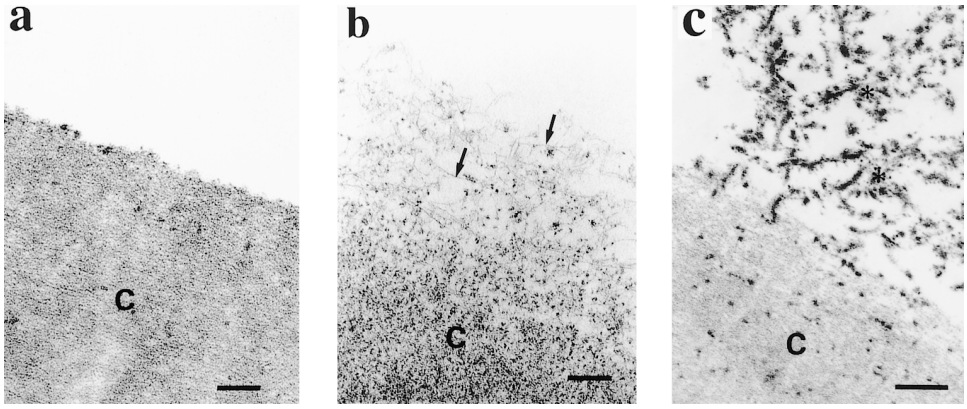

Figure 1. (**a**) Normal anterior capsular surface (c) with no visible fibers in 74-year-old woman (control). (**b**) Precapsular layer (arrow) seen on surface of anterior capsule (c) in 76-year-old exfoliation suspect. Thickness of precapsular layer (PCL) is 0.5–2.0 μm. (**c**) Specimen obtained from 70-year-old patient with clinical exfoliation syndrome. Exfoliation material (*), on surface of lens capsule (c), showing randomly arranged tangle of fibers (30–50 nm in diameter) embedded in amorphous ground substance. Bars: (**a** and **b**) 1 μm; (**c**) 0.5 μm.

Discussion

The pathogenic process of the exfoliation syndrome remains obscure in many of its aspects. Recognition of its early clinical and pathological changes could give clues to the mechanism of its production. Bartholomew[16] considered radial, peripheral, and gray striations of the anterior lens capsule to be the earliest biomicroscopic changes in the exfoliation syndrome and termed the condition "the pre-granular stage." Dark and Streeten[17] reported the presence of a subtle opacified surface layer on the anterior lens capsule in many older patients with and without exfoliation, and they were the first to report its ultrastructure as consisting of fibrils of 3–6 nm in width with a periodicity of 10–12 nm. They suggested that this precapsular film is the precursor of the exfoliation materials. Tetsumoto et al[7] found that the precapsular layer was composed ultrastructurally of microfibrils, amorphous material, and granular inclusions in patients with early exfoliation syndrome.

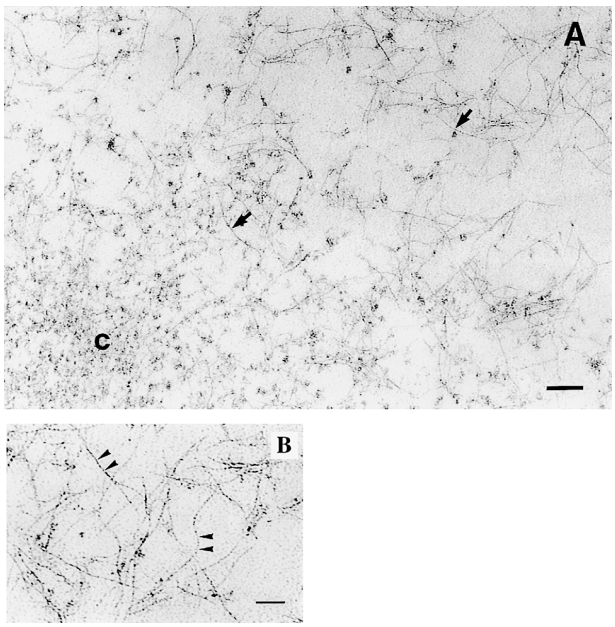

Figure 2. Specimen obtained from 76-year-old exfoliation suspect. (**A**) Electron microscopy revealed PCL was composed of fine network of microfibrils, 5–8 nm in diameter (arrows), amorphous material and granular inclusions with no clear demarcation between PCL and capsule (c). Bar = 1 μm. (**B**) Microfibrils have 12–14 nm periodicity (arrowheads). Bar = 0.1 μm.

In the present study, a precapsular layer was identified ultrastructurally on the lens surface in the eyes of 7 of 10 clinical exfoliation suspects and in 5 of 16 controls. This higher incidence of the precapsular layer in eyes of clinical exfoliation suspects than in control elderly subjects is similar to previously published data.[7] This suggests a clear correlation between lens capsular alterations and the clinical signs related to iris stromal atrophy and dispersion of pigment from the iris pigment epithelium. This is in accordance with previous reports[7] and confirms the relationship between the presence of a PCL and the development of exfoliation.

No clear explanation has been given for the development of the PCL. In this study, we have detected fragmented zonular fibers interspersed with and passing smoothly into the PCL microfibrils as well as

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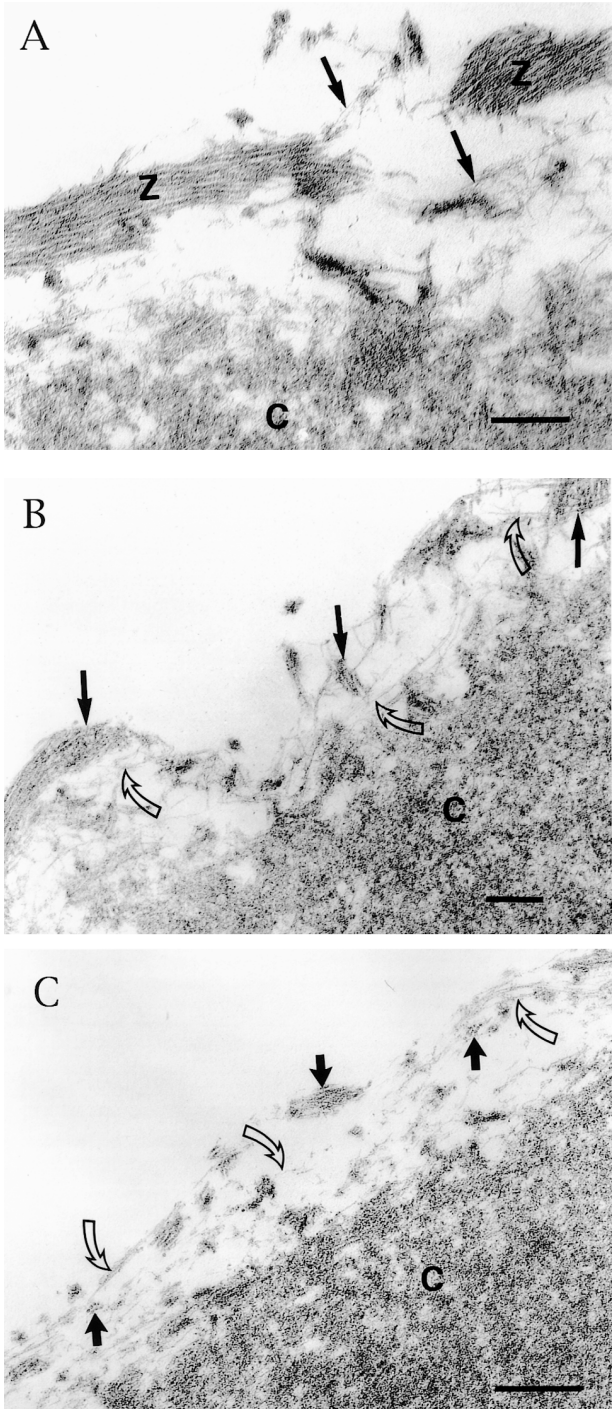

Figure 3. Specimens obtained from exfoliation suspects. Zonular fibrils are found closely related to and merging with PCL. c: capsule. (**A**) Exfoliation suspect, 73-year-old. Fragmentation of zonular bundles (z) with microfibrils in between (arrows). Bar = 0.2 μm. (**B**) and (**C**) Exfoliation suspects, 69 years old (**B**) and 72 years old (**C**). Remains of zonular fibers (black arrows) merge with microfibrils (open arrows) in PCL. Bar = 0.5 μm.

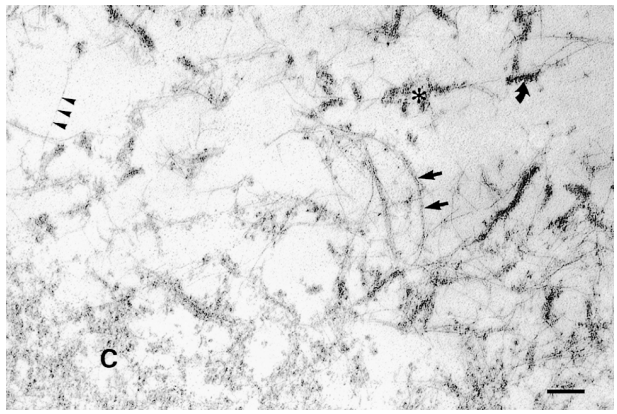

Figure 4. Specimen obtained from 45-year-old patient with exfoliation syndrome. Section shows two types of fibers, exfoliation fibers (curved arrow) and PCL microfibrils (arrowheads); latter sometimes merge forming thicker units (arrows), while others show deposition of electron-dense material with subsequent thickening (*). Bar = 1 μm.

in the fibrillary layer in areas free of exfoliation in exfoliative eyes. This implies that degeneration of zonular fibrils, which are recognized as microfibrils,[18–22] plays a role in the formation of these fibrillary matrices, especially close to the lens periphery. In support of this hypothesis is the diffuse positivity for fibrillin, a glycoprotein closely associated with zonules and other elastic tissue microfibrils[23,24] in the precapsular layer as reported by Dark and Streeten.[17] In fact, there is an increased extracellular deposition of fibrillin-containing fibrils in the exfoliation material on the anterior lens capsule and other tissues of the anterior segment.[25] The zonular fragments detected in this study were seen dispersed over the capsule more widely than what would be occasionally found in the peripheral portions of capsulotomy samples. In addition, no such fragmented zonular fibrils could be detected in normal control anterior capsules, minimizing the possibility that they are normally located zonular remnants, and suggesting that they are a part of a degenerative process in zonular fibers. Noteworthy, however, is that a fibrillary layer similar to the PCL has not been detected as yet on the posterior capsule to which zonular fibers are also inserted. This can be attributed to the difference in the aqueous humor dynamics and the nature of both interfaces, ie, anterior capsule-aqueous and posterior capsule-anterior hyaloid. The trapping of degenerated zonular fragments on and within an already existing PCL, which is known to contain laminin and other adhesion-promoting glycoproteins,[7] is still a

possibility, although this assumption also requires some zonular degeneration.

Several questions remain to be answered. Would degenerating zonules be the sole source for the PCL even in the central anterior capsule? As microfibrils are known to be produced by several cell types, including epithelial cells,[26] production by lens epithelial cells is still a possibility. We have observed fibrillary bands arising from the lens epithelial cells to the capsular surface in one capsule (not described). Secondary deposition from aqueous humor of microfibrils produced elsewhere is still another possibility, especially for those found on the central lens surface. We have previously detected microfibrils on the posterior surface of the iris in elderly eyes.[27] Whether this process of deposition implies deposition of microfibrils of zonular origin, or those produced by the cells lining the anterior chamber, or a local polymerization from precursors in the anterior chamber remains to be answered.

Supporting these conclusions is what has been suggested in several reports on the origin of exfoliation material with its established relationship to microfibrils. Abnormal aggregation of glycoproteins associated with the zonular-elastic microfibrillar system has been suggested in the pathogenesis of exfoliation syndrome.[13,15] Zonular fibrils, which are composed of microfibrils[18,20] were found in some reports to be disorganized and degenerated in eyes with exfoliation and were suggested to be a precursor of exfoliation.[11,14,28] Another report[10] has suggested that two processes are involved in the distribution of the exfoliation material. In the anterior ocular segment: (1) secondary deposition from the aqueous humor and (2) local production from the epithelial cells of the iris and ciliary body, pre-equatorial lens epithelium, corneal endothelium, and zonular fibrils.

In the present study, in the fibrillary layer seen in areas free of exfoliation aggregates in exfoliative eyes, many of the microfibrils were observed to be fused together, while others showed anterior chamber deposition of some electron-dense material and subsequent thickening, as if they were changing into thicker exfoliation fibers. Our findings support the hypothesis that exfoliation material can originate from microfibrils, and adds evidence to the direct role played by the precapsular layer in the development of exfoliation material.[15]

To conclude, fragments from degenerated zonular fiber might take part in the formation of the PCL microfibrils. Other possible sources include local production from anterior lens epithelial cells, and deposition of microfibrils produced elsewhere in the anterior chamber.

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