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Cellular Fibronectin, but not Collagens, Disappears in the Central Posterior Capsules During Healing After Lens Extraction and IOL Implantation in Rabbits

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Purpose: To investigate the nature of capsular opacification after cataract-intraocular lens (IOL) surgery in rabbit eyes, we immunohistochemically located extracellular matrix components in lens capsules after the surgery using light microscopy. The study was conducted also to compare the extracellular matrix components in rabbit capsules with those previously reported in the human eye.

Methods: Twenty-seven eyes of 17 Japanese albino rabbits were lensectomized by phacoemulsification, and IOLs were implanted. Using immunohistochemical methods, the lens capsules were examined immediately after surgery, and 1, 2, 4, and 8 weeks after surgery.

Results: In all cases at each time point, the edge of the anterior capsulotomy had contracted and was found to adhere to the inner surface of the posterior capsule, with both IOL haptics remaining in the capsular bag. Collagen types I and III were detected around the adhesion between the anterior capsulotomy edge and posterior capsule during all stages of healing and also observed on the central posterior capsules 1 or more weeks after surgery. Immunoreactivity for cellular fibronectin was seen around the adhesion between the anterior capsulotomy edge and posterior capsule during all stages of healing. It was also detected on the posterior capsules 2 and 4 weeks after surgery, but disappeared 8 weeks after surgery.

Conclusion: Extracellular matrix components such as collagen types I and III and cellular fibronectin were expressed inside the residual lens capsular bag. Cellular fibronectin may play a role in the early wound healing process in the postoperative posterior capsule because the immunoreactivity in the central posterior capsule disappears in the later phase of healing.
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Key Words: Cataract surgery, cellular fibronectin, collagen, immunohistochemistry, lens capsules.

Introduction

The occurrence of postoperative posterior capsular opacification following cataract-intraocular lens (IOL) surgery may result in the reduction of postoperative visual acuity and also in intraocular lens decentration caused by an abnormal capsular shrinkage.[1,2] Lens epithelial cell (LEC) proliferation and their extracellular matrix (ECM) production are responsible for this complication.[3,4] We have reported that posterior capsule opacification in human eyes with IOLs contains collagen types I, III, IV, V, and VI as well as fibronectin, laminin, proteoglycans and hyaluronan.[5–9] Moreover, we have reported that postoperative rabbit lens epithelial cells express prolyl 4-hydroxylase, an enzyme involved in collagen biosynthesis, similar to what occurs in human eyes, in

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association with ECM deposition.[10] The tissue reaction after cataract extraction is, in part, the wound healing reaction performed by LECs.[11] However, the constituents of ECM accumulated in the posterior capsular opacification of experimental animals have not been well examined. Morphological studies indicate the presence of collagen and proteoglycan.[5]

The present study was undertaken to examine the presence and distribution of collagen types I and III, as well as cellular fibronectin (cFN), in post cataract—IOL surgery lens capsules in rabbits. The purpose of this study was to develop an animal model of posterior capsular opacification and also to compare the ECM components of this opacification in the rabbit with those previously reported in human eyes.[6,7]

Materials and Methods

Surgery in Rabbits

We used 27 eyes of 17 adult Japanese albino rabbits (2.5 kg body weight). Surgical procedures were the same as previously reported.[5,10] The animals were anesthetized with intramuscular injection of ketamine hydrochroride (50 mg/kg) and xylazine (10 mg/kg). Intercapsular phacoemulsification was performed and the remaining cortex was aspirated. After the insertion of a polymethyl methacrylate IOL into the capsular bag, a circular window was made in the anterior capsule using a forceps.

Immunohistochemistry

The rabbits were sacrificed by an intravenous injection of an overdose of pentobarbital sodium either immediately (5 globes) or at 1 (8 globes), 2 (5 globes), 4 (5 globes), or 8 (4 globes) weeks after the surgery. The eyes were enucleated, hemisected along the equator, and the corneas were removed. The IOL was delicately removed from the capsular bag. The anterior half of the eye was embedded in OCT compound (Miles, Elkhart, IN, USA). Cryosections (5 μm thick) were fixed with cold acetone and processed for indirect immunostaining as previously reported.[8] The primary antibodies used were as follows: a monoclonal anti-cFN antibody [× 400 in phosphate-buffered saline (PBS); Sigma, St. Louis, MO, USA], a monoclonal anti-collagen type I antibody (× 100 in PBS) and a monoclonal anti-collagen type III antibody (× 100 in PBS). Both anti-collagen antibodies were donated by the Department of Pathology, Wakayama Medical College, Japan. The secondary peroxidase-conjugated antibody against mouse immunoglobulin was obtained from Cappel-ICN (Organon-Teknika, West Chester, PA, USA) (× 100 in PBS). Antibody complex was visualized with 3,3′-diaminobenzidine, as previously reported.[8]

Results

In all cases at each time point, the edge of the anterior capsulotomy was contracted and was found to adhere to the inner surface of the posterior capsule, with both IOL haptics remaining in the capsular bag. Neither collagen types I (Figure 1A) nor III (not illustrated), nor cFN (Figure 3A) was found in the lens capsule immediately after surgery. All three proteins were found inside the lens capsules at 1, 2, 4, and 8 weeks after surgery, although the distribution pattern was dissimilar.

One week after surgery, anterior and posterior capsules in the equatorial region were found to attach to each other without forming regenerated lenticular fibers. The LECs there were negative for collagen types I and III and cFN. At that time, collagen types I (Figure 1B) and III (not illustrated) and cFN (Figure 3B) were detected in the area where the residual anterior capsular edge was found to attach to the posterior capsule in all specimens examined. They were observed on the central posterior capsule (in the area circled by the anterior capsule-posterior capsule adhesion) in 3 of the 8 specimens examined at 1 week after surgery (Figures 1C and 3B).

Two or four weeks after surgery, collagen types I and III, as well as cFN, were detected not only in the area of fusion between the anterior capsular edge and the posterior capsule, but also on the inner surface of the central opacified area in all specimens examined (Figures 1E,F, 2, 3D). The equatorial region of the capsular bags was completely filled by the regenerated lenticular fibers, forming Soemmerring's ring. The regenerated lens fibers were negative for collagen types I (Figure 1D) and III (not illustrated) and cFN (not illustrated).

Eight weeks after surgery, the distribution pattern of collagen types I (Figures 1G,H) and III (not illustrated) were similar to that 2 or 4 weeks postoperatively. cFN was, however, not detected on the inner surface of the central opacified area (in the area circled by the anterior capsule-posterior capsule adhesion), while it was there in the matrix accumulation around the adhesion of the anterior and posterior capsules (Figures 2E,F; Tables 1 and 2).

No specific immunoreactivity was seen in the negative control staining performed with the omission of each primary antibody.

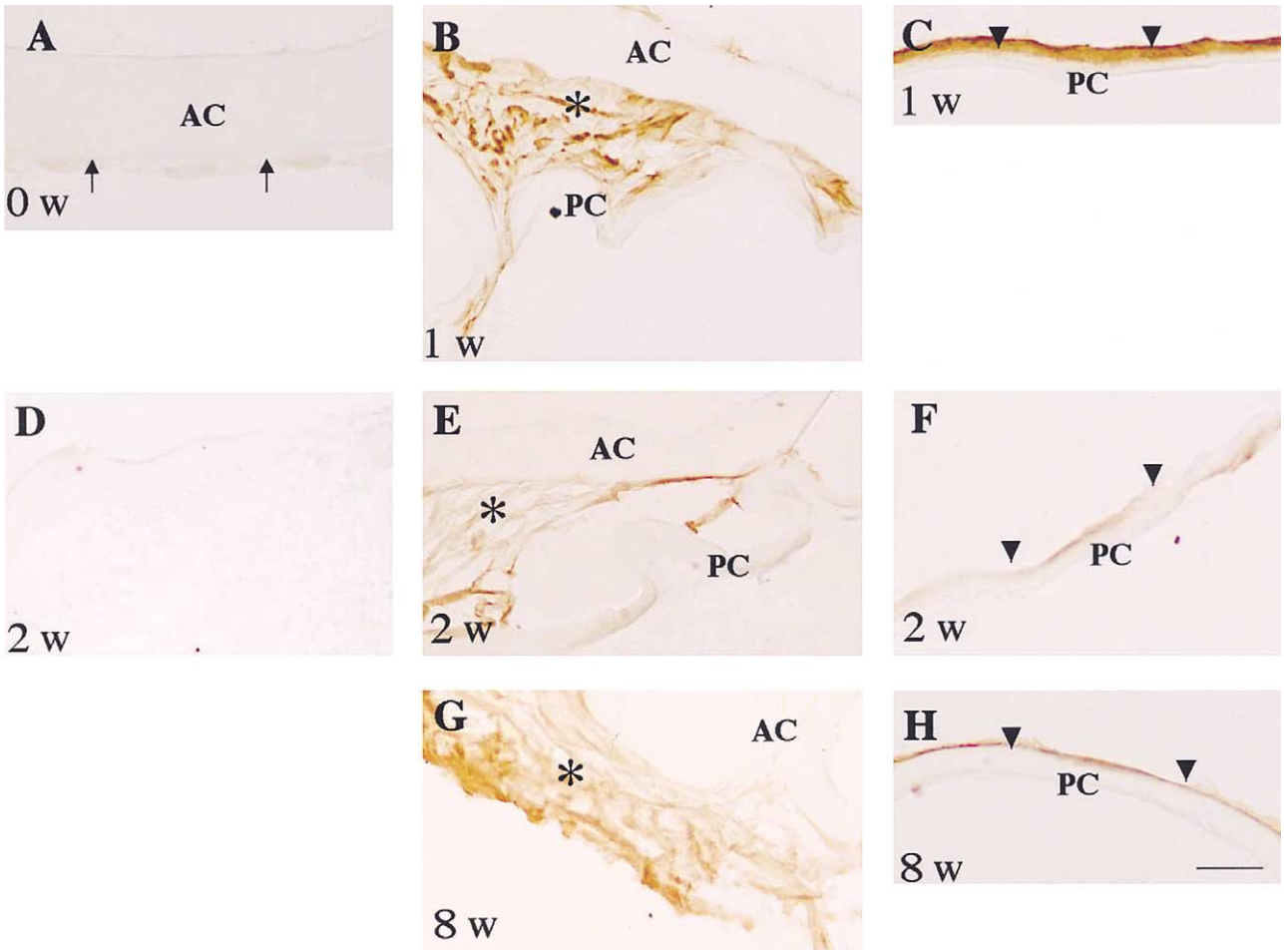

Figure 1. Immunolocalization of collagen type I in rabbit lens capsules at various postoperative healing intervals. (**A**) Immediately after the operation, lens epithelial cells (arrows) do not stain for collagen I. Throughout the healing intervals, this collagen type is immunohistochemically detected both in the matrix accumulation around the adhesion of anterior and posterior capsules (asterisks, **B**, **E**, **G**), as well as on the central capsule (arrowheads, **C**, **F**, **H**). Lens cells in the Soemmerring's ring are negative for collagen I (**D**). (**B**) and (**C**), 1 week postoperatively; (**D**–**F**), 2 weeks postoperatively; (**G**) and (**H**), 8 weeks postoperatively. Indirect immunostaining. AC: anterior capsule, PC: posterior capsule. Bar: 20 μm (**A**), 50 μm (**B**–**H**).

Discussion

In the present study, collagen types I and III, as well as cFN, were found to be expressed in the posterior capsular opacification after lens extraction and IOL implantation in rabbits.

The distribution pattern of the collagens was similar to that observed in human eyes previously reported.[6–9] Collagen types I and III were expressed as early as 1 week after surgery in the adhesion site of the anterior capsular edge and posterior capsule of 3 of the 8 specimens examined at this time. LECs are considered to transform their phenotype into the fibroblastic type so that these cells are responsible for the accumulation of ECM components inside the lens capsular bag.

Fibronectin (FN) was also expressed similarly to collagens in the posterior capsular opacification in earlier stages of healing. cFN was, however, no longer detected on the central posterior capsule (in the area circled by the anterior capsule-posterior capsule adhesion) whereas collagens were seen there 8 weeks after surgery. cFN therefore may play a role in the capsular repair process in a relatively early phase in rabbits. Similar expression patterns of cFN during wound healing were observed in various ocular tissues, ie, corneal wound healing.[12] In vitro study showed that FN promotes the adhesion and migration of rabbit lens epithelial cells.[13] Inhibition of cFN activity may be effective in inhibiting the occurrence of the posterior capsular opacification. In vitro study

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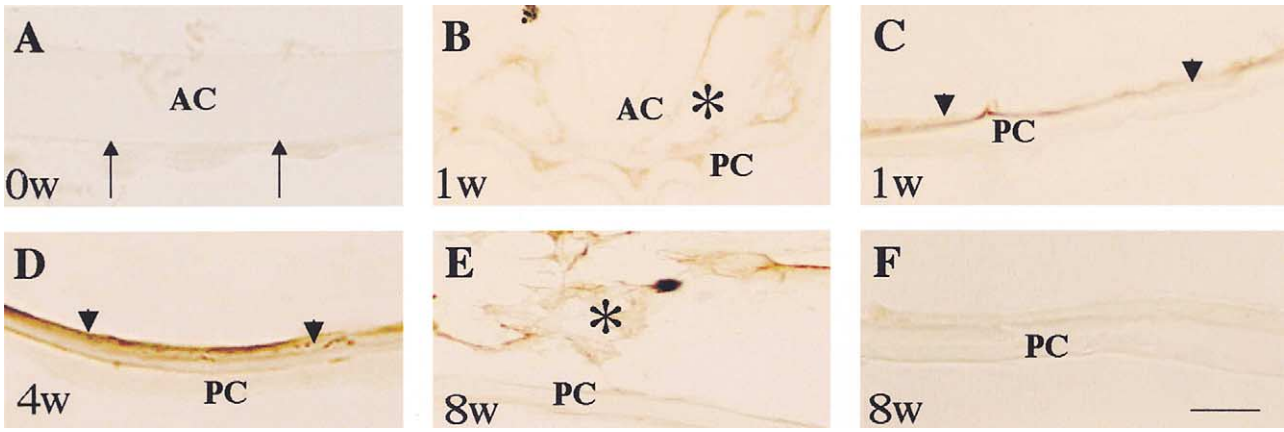

Figure 2. Immunolocalization of cellular fibronectin in rabbit lens capsules at various postoperative healing intervals. (**A**) Immediately after the operation, lens epithelial cells (arrows) do not stain for cellular fibronectin. Throughout the healing intervals, cellular fibronectin type is immunohistochemically detected in the matrix accumulation around the adhesion of the anterior and posterior capsules [asterisks, (**B**), (**E**)]. Matrix accumulated on the central posterior capsule is positive for cellular fibronectin up to 4 weeks postoperatively (arrowheads, **C**, **D**), but is not seen at 8 weeks postoperatively (**F**). (**B**) and (**C**), 1 week postoperatively; (**D**), 4 weeks postoperatively; (**E**) and (**F**), 8 weeks postoperatively. Indirect immunostaining. AC: anterior capsule, PC: posterior capsule. Bar: 20 μm (**A**), 50 μm (**B**–**F**).

also showed that the blockage of cFN activity by cFN-derived oligopeptides reduced the attachment of lens epithelial cells.[14] FN accumulated on the central posterior capsule is considered to be degraded by matrix metalloproteinases (MMP), because we have detected MMP expression in fibrotic human lens capsules extracted within 18 months after IOL implantation, but not in the specimens examined 24 or more months after surgery.[15] cFN deposited in the organized matrix located in the space between anterior and posterior capsule might be more resistant to MMP degradation than that deposited on the posterior capsule not covered by the anterior capsule. Because FN provides a provisional matrix scaffold for cell movement,[12] disappearance of cFN on the central capsule during the later phase of healing might disturb the migratory activity of LECs there. We have previously reported that cFN was detected 9 and 19 months after IOL implantation in capsules extracted from the eyes of patients with diabetic retinopathy or proliferative vitreoretinopathy.[8] The exact reason for this discrepancy in the cFN expression pattern is unknown. Growth factor levels in human eyes with proliferative diseases are higher than in normal eyes,[16,17] presumably influencing the phenotypic alteration in the residual lens epithelial cells.

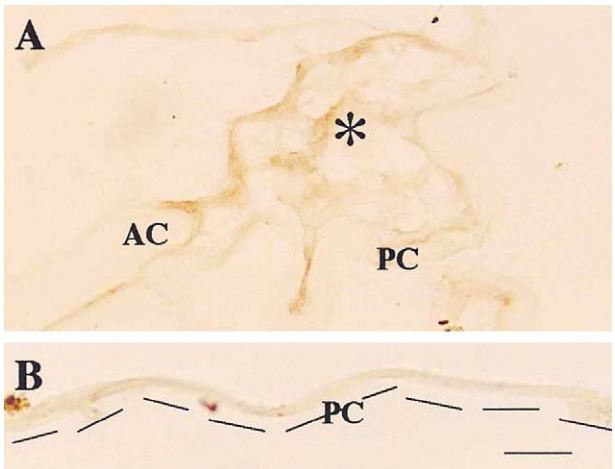

Figure 3. Immunolocalization of collagen type III in rabbit lens capsules at 8 weeks postoperatively. This collagen type is immunohistochemically detected both in the matrix accumulation around the adhesion of anterior and posterior capsules (**A**), as well as on the central capsule (**B**). Dotted line, posterior surface of the posterior capsule. Indirect immunostaining. Bar: 50 μm.

Table 1. Extracellular Matrix Accumulation in Area of Adhesion of Anterior Capsulotomy Edge and Posterior Capsule

	Time After Surgery (Weeks)				
ECM Accumulated	0	1	2	4	8
Collagen type I	−	+	+	+	+
Collagen type III	−	+	+	+	+
Cellular fibronectin	−	+	+	+	+

ECM: extracellular matrix, +: positive, −: negative.

Table 2. Extracellular Matrix Accumulation on Posterior Capsules

	Time After Surgery (Weeks)				
ECM Accumulated	0	1	2	4	8
Collagen type I	−	+/−	+	+	+
Collagen type III	−	+/−	+	+	+
Cellular fibronectin	−	+/−	+	+	−

ECM: extracellular matrix, +: positive, +/−: positive in 3 of 8 specimens, −: negative.

We have reported the up-regulation of AP-1 components, ie, c-fos and c-jun, and the nuclear translocation of Smads 3/4 in epithelial cells of rat or mouse injured lens during healing.[11,18] In these reports, expression of AP-1 was transient,[18] but Smads nuclear translocation was occasionally observed in fibroblastic lens cells even in the later phase of healing.[11] FN up-regulation by transforming growth factor-β is reportedly AP-1-dependent, but Smad-independent.[19] LEC behavior in the formation of posterior capsular opacification is also a part of the wound healing of the lens tissue. Transient up-regulation of AP-1 in LECs in an injured animal lens might therefore account for the disappearance of cFN in the later phase of PCO formation.

cFN reportedly regulates the epithelial-mesenchymal transition in many cell types.[20,21] The LECs between the anterior and posterior capsules in the equatorial region were considered to keep the original LEC phenotype without expressing collagens and cFN and then to form a regenerated lenticular structure. Because, in the present study, we enlarged the defect in the anterior chamber after implantation of an IOL into the capsular bag, the optic part of the IOL was found to be located outside the capsular bag while both haptics were in the capsular bag. In this situation, the capsular bag was closed and the wound healing reaction might have been minimally influenced by the presence of the IOL; the cells might have been able to survive apart from the continuous irritation by the foreign body. This might have reduced the activity of lens epithelial cells in the later phase of capsular healing at 4 weeks postoperatively.

Inhibitors for prolyl 4-hydroxylase or lysyl hydroxylase, both key enzymes involved in collagen biosynthesis, reduce collagen production as well as cell proliferation in fibroblasts in vitro.[22–24] Drugs with an inhibitory effect on cytokine action or collagen biosynthesis[22–25] can inhibit collagen biosynthesis in vivo, and this can be a new strategy for inhibiting the formation of posterior capsule opacification. The present study successfully developed a rabbit model of this opacification, which may be useful in investigating the inhibitory effects of such drugs in vivo.

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