The Proteinaceous Coating and Cytology of Implant Lenses in Rabbits

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We performed extracapsular lens extraction with implantation of a J-loop posterior chamber lens in 14 rabbit eyes. Postoperatively, the animals were examined by slit lamp. They were killed after varying survival times of up to 12 weeks, and the implants were examined by scanning electron microscopy. Three days after the operation a thin amorphous coating that did not consist of collagen was found covering all implants. Three cell types were present on the coating: macrophages, leukocytes, and flattened giant cells. This coating resembled morphologically the fibronectin coating on intraocular lenses *in vitro*.

More than 500,000 intraocular lenses are implanted per year in the United States.¹ The most widely used implant materials (polymethylmethacrylate, polypropylene, and polyamide) show a generally good biocompatibility.² The question has been raised why these artificial materials do not induce a destructive foreign body reaction.³ However, a mild reaction of the host to the implanted lens has been described in humans.⁴⁻⁷

To investigate this phenomenon systematically we used an animal model. We implanted posterior chamber intraocular lenses in 14 rabbits. The animals were killed according to plan after varying survival times. Histologic

findings on the surface of the implant were studied by scanning electron microscopy to determine the sequence of events in the reaction of the host to the implant.

Material and Methods

Fourteen sodium hydroxide sterilized polypropylene looped polymethylmethacrylate lenses (J-loop type) were implanted after extracapsular lens extraction in the eyes of adult Dutch pigmented rabbits. Preoperatively the rabbits were treated for three days with 0.5% indomethacin eyedrops three times a day to prevent fibrin accumulation during intraocular surgery. During the two hours before surgery 10% phenylephrine, 1% cyclopentolate, 1% tropicamide, and 0.5% indomethacin eyedrops were administered every 15 minutes to dilate the pupils. Shortly before surgery the animals were sedated by intramuscular injection of 2 ml of chlorpromazine (5 mg/ml) and anesthetized by slow injection of 2 to 4 ml of pentobarbital sodium (60 mg/ml) in the marginal ear vein. Analgesia was improved by subconjunctival injection of 1 to 2 ml of prilocaine hydrochloride (20 mg/ml). All operations were performed under a microscope with coaxial illumination according to standards used in human cataract surgery by one of us (J.H.P.). Both eyes of each animal were operated on.

A bent, disposable 27-gauge needle on a syringe filled with air was stabbed through the cornea without prior incision. With this needle the anterior lens capsule was opened along the pupillary border. A 150 to 160 degree corneal incision was made. Two corneal sutures were placed for security and were looped aside. The anterior capsule was grasped with a pair of Vogt capsule forceps. The lens nucleus was easily washed out by positive vitreous pressure with an olive cannula and Ringer's solution.

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However, mild expression was necessary in some cases. The residual cortical material was irrigated using a flat cannula, although hyperemia of the iris and narrowing of the pupil prevented complete irrigation. The anterior chamber was then restored by injection of 1 ml of sodium hyaluronate. Insertion forceps were used to grasp the superior haptic of the intraocular lens, and the inferior haptic was slipped into place in the inferior capsular fornix. By rotating the lens the superior haptic was brought into the superior capsular fornix. To prevent bleeding and excessive fibrin formation no peripheral iridectomy was performed. The corneal incision was closed in one layer using 10-0 nylon.

No postoperative treatment was given. During the first postoperative day we observed a mild to severe fibrinous reaction in the anterior chamber and the occurrence of pigmented precipitates on the optic part of the implant. The dissolution of the fibrin and the development of cellular elements on the implant was tracked by slit-lamp examination.

After survival times varying from three days to 12 weeks the animals were injected with an overdose of pentobarbital sodium (Table). The eyes were immediately enucleated and placed in a cacodylate buffered glutaraldehyde/formaldehyde fixative.⁸ To improve penetration of the fixative the anterior chamber was opened by a corneal incision. After one week of fixation the anterior segments were thoroughly rinsed in cacodylate buffer, dehydrated in a graded series of ethanols, and critical-point dried with CO₂.⁹ The specimens were glued on specimen stubs with conductive carbon cement and coated with approximately 7 nm of gold. The specimens were examined by scanning electron microscopy using a secondary electron detector.

Additional experiments with three new, wet-pack sterilized J-loop intraocular lenses were

performed. One lens was incubated at 37 C for 24 hours in saline, and two lenses were incubated separately at 37 C for 24 hours in a solution of 1,000 µg/ml of fibronectin. After fixation in cacodylate buffered glutaraldehyde/formaldehyde fixative⁸ the lenses were prepared for scanning electron microscopy as described above.

Results

On the first postoperative day slit-lamp examination through the somewhat hazy cornea disclosed varying amounts of fibrin together with a moderate number of pigmented deposits on the anterior surface of the lens. During the first two weeks postoperatively most of the macroscopically visible fibrin disappeared and more pigmented deposits could be seen on the optic. The iris showed a mild to severe hyperemia, and in three cases pupillary capture occurred. In one case the fibrin reaction was severe and an irregular mass almost filled the anterior chamber. Since this animal was to be killed three days after the operation, we did not wait for the dissolution of the fibrin. The contralateral eye of this animal showed almost no fibrin reaction.

As the survival time increased, the size of the pigmented deposits increased. The maximum number of deposits (ten to 15) was present about three weeks after the operation and could be seen on the anterior side of the implant. Dissection of the eyes showed that in all cases both loops of the intraocular lens were placed in the capsular bag and that the peripheral cortical remnants were enclosed by the anterior capsular flap and the posterior capsule, forming a Soemmerring's ring.

Scanning electron microscopy disclosed the ultrastructure of the pigmented deposits on the anterior surface of the lens (Fig. 1). Two intraocular lenses with an implantation time of three days were studied. On one, a dense mass of fibrin and a number of leukocytes were present. The fibrin mass prevented further inspection of the lens surface. The other specimen showed less fibrin. On the surface of this lens a thin, wrinkled coating was found, which was accidentally torn at several places (Fig. 2). The thickness of this coating did not exceed 1 µm. On this coating numerous cells were distributed over the entire surface. More than 100 cells were identified, ranging in size from

TABLE
NUMBER OF RABBIT EYES IMPLANTED WITH
INTRAOCULAR LENSES AND THEIR SURVIVAL TIMES

NO. OF EYES	SURVIVAL TIME
2	3 days
2	1 wk
2	2 wks
6	4 wks
2	12 wks

Fig. 1 (Kappelhof and associates). By scanning electron microscopy, at one week survival time, anterior view of posterior chamber intraocular lens in the rabbit eye with the cornea dissected. Cells on the anterior surface are visible. The iris is indicated by the arrow ($\times 41$).

Fig. 2 (Kappelhof and associates). At three days' survival time, a thin, amorphous coating, accidentally torn at several places (arrowheads) was found on the anterior surface of each implanted intraocular lens. Cells were found on the coating (arrow) ($\times 680$).

Fig. 3 (Kappelhof and associates). At three days survival time a small number of large cells with a diameter up to 100 μm were found on the anterior surface of the implant (arrowheads). The arrows indicate the prevalent, smaller cell type ($\times 442$).

Fig. 4 (Kappelhof and associates). On the surface of the implant at one week survival time different cell types could be distinguished. The smallest cell type, about 5 μm in diameter, is indicated by arrows. The larger cell type, indicated by an arrowhead, was round and flat with a central elevation and a diameter between 10 and 30 μm ($\times 1,770$).

10 to 100 μm . Most of the cells were flat, had a round to oval outline (maximum diameter, 10 μm), and were covered with villi (Figs. 2 and 3). Only few large flat cells were found with diameters up to 100 μm (Fig. 3).

The findings one week postoperatively were similar to the findings three days postoperatively. However, three different types of cells were identified. The first type of cells, approximately 5 μm in diameter, were spherical and covered with villi (lymphocytes) (Fig. 4). The second type of cells were round and flat with a central elevation and a diameter between 10 and 30 μm . They were probably activated macrophages (Fig. 4). The third cell type was large, irregularly shaped, and had a maximum diameter of 150 μm (Fig. 5). The two specimens studied here were both covered with several hundreds of cells of which only 10% were of the third type. The remaining 90% consisted of about equal numbers of the first and second types of cells. There was still some fibrin present (Fig. 5).

Two weeks postoperatively the thin coating on

the lens was still present together with some fibrin and numerous cells of the three types described above. However, the large cells were even larger. Flattened cells, covered with microvilli, and over 300 μm wide, were found near the holes in the optic part of the intraocular lens that are used for manipulation during surgery (Fig. 6). Other cells, more than 100 μm in diameter, were round and showed a central elevation.

Four weeks postoperatively the coating on the lens was still present and in some cases fibrin was found. The cell population was essentially the same as that found three weeks postoperatively, including the huge, flattened cells. Few huge cells were seen in each specimen, but their size exceeded 400 μm . As can be seen in Figure 7 they had growing processes and were incomplete in covering the corresponding surface area.

Only one specimen was examined by scanning electron microscopy after 12 weeks' survival time. The coating on the implants was still present. There were fewer cells on the surface

Fig. 5 (Kappelhof and associates). At one week survival time a small number of huge cells was found on the anterior surface of the implant, representing the third identifiable cell type. The arrow shows the location of fibrin ($\times 600$).

Fig. 6 (Kappelhof and associates). At two weeks' survival time some large cellular elements were found near the manipulation holes of the implant. They were flat, covered with microvilli, and over 300 μm wide ($\times 250$).

Fig. 7 (Kappelhof and associates). One of the round, large cells with a central elevation (arrow) found on the anterior surface of the implant at two weeks' survival time ($\times 570$).

Fig. 8 (Kappelhof and associates). Some large cellular elements were found near the implantation site of the haptics at 12 weeks' survival time ($\times 90$).

of the lens, and most of them were found in the periphery. The center of the optic was almost free of cells. In the periphery fibrin was found together with leukocytes. Large cells, exceeding $350\text{ }\mu\text{m}$ in size, were found near the place of implantation of the haptics (Fig. 8) and in and near the holes in the optic part of the lens.

The other specimen with 12 weeks' survival time was used for experiments concerning the nature of the thin coating on the lens. The implant was dissected from the eye immediately after death and divided into three parts: one part was treated with collagenase, one part with trypsin, and one part with cationized ferritin. Scanning electron microscopy of these parts disclosed a coating on the collagenase-treated part, comparable to that on untreated lenses, and the absence of this coating on the trypsin-treated part. Without gold-coating and with the use of a low accelerating voltage the cationized ferritin-treated part of the lens showed a thickened layer of electron conducting material, indicating the presence of iron bound to the surface of the lens.

Scanning electron microscopy of the unused intraocular lens incubated at $37\text{ }^{\circ}\text{C}$ for 24 hours in saline disclosed a clear surface without any coating. Examination of the two intraocular

lenses incubated separately in a solution of fibronectin ($1,000\text{ }\mu\text{g/ml}$) at the same temperature and for the same period of time, showed a thin, wrinkled coating resembling the coating found on the rabbit implants (Fig. 9).

Discussion

Experimental surgery performed in rabbits has some major differences when compared with human surgery. The fibrin accumulation in rabbits during intraocular surgery is well known and has no counterpart in humans. However, the reaction described in this study might be applicable to humans since pigmented deposits, cellular elements, and membranes have been frequently reported findings in humans.³⁻⁷

As discussed by various authors,⁴⁻⁷ the implantation of an intraocular lens in humans causes a limited foreign body reaction. Wolter⁵⁻⁷ described a thin proteinaceous layer with numerous cells on the surface of intraocular lenses obtained from humans postmortem or after surgical removal.

The membranes on intraocular lenses de-

Fig. 9 (Kappelhof and associates). An unused intraocular lens was placed in an aqueous solution of fibronectin (1,000 $\mu\text{g}/\text{ml}$) for 24 hours. Scanning electron microscopy disclosed the presence of a thin, amorphous coating resembling the coating found in vivo as seen in Figure 2 ($\times 500$).

scribed by Wolter are partly cellular with an acellular proteinaceous component that fills the interspaces.⁵ These human specimens had a longer implantation time than the lenses in our study. The cells observed were primarily macrophages or differentiation products of macrophages, such as epithelioid cells and giant cells. Almost all of these cells contained pigment granules in their cytoplasm. These findings were confirmed by Sievers and von Domarus.⁴ In 1986, the scanning electron microscopic finding of a thin wrinkled coating on the intraocular lens was described in human material.¹⁰

The three cell types found in this study correspond to those previously reported. The smallest cells are apparently leukocytes, present as a result of the wound reaction. The larger, round cells with the central elevation are macrophages, and the third cell type consists of either epithelioid cells or giant cells. Differentiation between these two latter types is difficult by scanning electron microscopy.

The most remarkable finding in our study is the rapid appearance (three days postoperatively) of a thin coating on the lens. Ohara³ reported in vivo large pigmented deposits and fine pigment particles on the surfaces of an

implanted lens in humans from the first post-operative day in an uncomplicated case.

To determine the nature of this coating we treated it with collagenase, trypsin, and ferritin. Since collagenase did not harm the coating, the coating was not made of collagen. Trypsin, a protein digesting enzyme, destroyed the coating. Ferritin, which binds to acid groups of proteins, did bind to the coating. The coating must, therefore, consist of a protein with acidic-binding areas.

We suspected that the coating might consist of plasma fibronectin. Fibronectin is a large glycoprotein that exists in both a cellular and a plasma form.¹¹ The cellular form is the major cell surface glycoprotein of many fibroblastic cell lines.¹² The plasma form of fibronectin has a lower molecular weight and is an extracellular protein. It has been demonstrated to mediate several cellular functions, namely cell attachment to substrates,¹³ maintenance of cellular structure,¹⁴ and nonimmune opsonization.¹⁵ Polymethylmethacrylate has been shown to bind and surface-activate fibronectin,¹¹ making it a potentially suitable substrate for fibronectin-mediated cell adhesion. Fibronectin can be released in wound reaction and is known to be produced by macrophages.¹⁶

In this study we show that plasma fibronectin produces in vitro a morphologically identical coating on intraocular lenses as the coating found experimentally in vivo in the rabbit. This observation supports the hypothesis that, shortly after surgery, plasma fibronectin is released in the aqueous and binds to the polymethylmethacrylate of the lens optic. Biochemical analysis of the thin coating on intraocular lenses will be necessary to prove this hypothesis.

To our knowledge, the presence of fibronectin in rabbits has not been previously reported and it might be an important contributor to cell growth on intraocular lenses. It is known to promote cell attachment and spreading. Fibronectin might contribute to the final acceptance of the implant by the eye.

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