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EVALUATION OF THE IMAGES OF SPECULAR MICROSCOPY OF THE CELLS ON IMPLANTED INTRAOCULAR LENSES IN VIVO

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(Received for publication October 11, 1990, and in revised form February 24, 1991)

Abstract

Specular microscopy, an optical method to study the anterior segment of the eye, was applied to observe the cells on implanted intraocular lenses in vivo. The images from specular microscopy were evaluated by comparing the morphology of the same cells on intraocular lenses implanted in rabbit eyes by specular microscopy, light microscopy and scanning electron microscopy (SEM). The time lapse study in living human eyes and the optical evaluation of its interference images were performed to elucidate its image formation.

The same giant cells, as observed in vivo by specular microscopy, proved to be multinucleated cells. It was demonstrated that, although the lamella of the cells contributed to specular microscopic images, filamentous extensions were not visible.

The complex images of concentric fringes and uniform gray areas on giant cells recognized by specular microscopy were interpreted to be generated by interference phenomena originating from both the cell cytoplasm and the separation between the cell and the intraocular lens, respectively. In addition, shadow-cast images of small round cells and fibroblast-like cells were noted. The application of specular microscopy as a form of interference reflection microscopy was used to study the cell-intraocular lens interaction in living human eyes.

KEY WORDS: Specular microscopy, intraocular lenses, multinucleated giant cells, interference images, shadow-cast images, scanning electron microscopy, diffraction, interference reflection microscopy.

Introduction

Intraocular lenses as biomaterials are not inert in the inner eye but lead to a cellular reaction on the surface of the implants (Wolter, 1982, 1985; Sievers and von Domarus, 1984; Bryan III et al., 1985; Kappelhof et al., 1986; Wenzel et al., 1987; Versura et al., 1987; Uenoyma et al., 1988; Ishibashi et al., 1989; Okada and Sagawa, 1989). It has been shown that the main cellular elements observed on implanted intraocular lenses are macrophages, fibroblast-like cells (sessile macrophages) and multinucleated giant cells (Wolter, 1982, 1985), and result from a foreign body reaction of the host to implanted intraocular lenses (Wolter, 1982, 1985; Sievers and von Domarus, 1984; Bryan III et al., 1985; Kappelhof et al., 1986; Wenzel et al., 1987; Versura et al., 1987; Uenoyma et al., 1988; Ishibashi et al., 1989; Okada and Sagawa, 1989). The foreign body reaction on intraocular lenses is different from those observed on the biomaterials implanted in other parts of the body, in that, intraocular lenses are tolerated without encapsulation by fibrous tissues. There is only a membranous coating, suggested to be caused by adsorption of proteins from the aqueous humor over the surface of the implants (Kappelhof et al., 1986; Okada and Sagawa, 1989).

The studies on the cellular reaction on explanted intraocular lenses have been performed independently by light microscopy (Wolter, 1982, 1985; Uenoyma et al., 1988), scanning electron microscopy (SEM) (Sievers and von Domarus, 1984; Bryan III et al., 1985; Kappelhof et al., 1986; Uenoyma et al., 1988) and transmission electron microscopy (Ishibashi et al., 1989).

As an in vivo method, specular microscopy was introduced to study the cells on intraocular lenses by Oak et al., (1983). This technique was first developed to observe corneal endothelium (Maurice, 1968) and then applied to study the other parts of the anterior segment of the eye such as corneal epithelium (Maurice, 1974; Oak et al., 1983; Serdarevic and Koester, 1985), tear film (Serdarevic and Koester, 1985), lens epithelium (Bron and Matsuda, 1981; Laing and Bursell, 1981) and the cells on intraocular lenses (Oak et al., 1983; Ohara, 1985; Wenzel et al., 1987; Okada and Sagawa, 1989;
Okada and Abe, 1989).

From an optical point of view, the principle of specular microscopy is to differentiate the interfaces of different refractive indices (Laing et al., 1979). The name "specular microscope" is derived from the optical mechanism by which images are generated from reflected light at interfaces, in a specular fashion; the angle of reflection at the interfaces is equal to the angle of incidence (Laing and Bursell, 1981). However, some images of the cells on intraocular lenses did not appear to originate from the intensity of reflected light which depends on the difference between the refractive indices of the two media forming an interface, as described on corneal endothelium (Laing et al., 1979). Indeed, there is no definite description on the image interpretation of the cells on intraocular lenses. Thus, we have attempted to answer the questions concerning the image formation of cells on intraocular lenses, using specular microscopy. In our preliminary report, we (Okada and Abe, 1989) suggested that the colors observed on giant cells on intraocular lenses are generated by interference phenomena and that these interference fringes arise from the cell cytoplasm.

In this study, to appropriately interpret the images of specular microscopy, we evaluated the morphological correlation between the images of the same cells on intraocular lenses by using specular microscopy, light microscopy and SEM. We also discuss the optical principle of this method which generates complex interference images of the cells. We interpret the optical events observed on the cells on intraocular lenses in the same manner as those of interference reflection microscopy. We also present the application of specular microscopy (as an interference reflection microscopy technique) to study cell-intraocular lens interaction in vivo. In addition, we demonstrate the shadow-cast images of small round cells and fibroblast-like cells.

Materials and Methods

Animal experiments

Albino and pigmented rabbits were kept under routine laboratory conditions. Before intraocular lens implantation, rabbits were injected subcutaneously with a combination of ketamine hydrochloride (Ketalar®) (50 mg/kg) and xylazine hydrochloride (Seractal®) (5 mg/kg). A 7.0 mm corneal incision was made in the upper part of corneal limbus and sodium hyaluronate (Healon®) was injected in the anterior chamber of rabbit eyes. Posterior chamber intraocular lenses (manufactured by Menicon Company, Japan) having 6.5 mm polymethylmethacrylate optics with polyvinylidenfluoride (PVDF) haptics, were implanted in the anterior chamber without extraction of lenses. The corneal incision was closed by 9-0 silk, interrupted sutures after the intraocular lens insertion. Cycloplegic mydriatic (Mydrin P®) and antibiotics were prescribed once a day for a week, postoperatively. After various survival times from two to four weeks after intraocular lens implanta-

tion, we studied the cells on the intraocular lenses in vivo by specular microscopy and then explanted the intraocular lenses for SEM and light microscopy. To avoid damages and artifacts, the intraocular lenses were explanted by grasping one of the haptics of the implants with a forceps. The same cells as observed by specular microscopy were first identified by light microscopy after staining with hematoxylin-eosin, and then were studied with the SEM.

Specular microscopy

Figure 1 shows a schematic diagram of the anterior or segment of the eye which contains an intraocular lens. The images of specular microscopy are generally formed by reflected light waves at interfaces of different refractive indices. When we study the cells on an intraocular lens, the light projected through the lower slit of the microscope, passing through the cornea, encounters interfaces such as the aqueous humor-cell interface, the cell-aqueous humor interface, and the aqueous humor-intraocular lens interface. Part of the incident light is reflected at the interfaces and is collected through the upper slit of the microscope to form an image.

Before specular microscopy, a cone lens, usually used to see the corneal endothelium, was taken off from the microscope and an interference filter, which reduces the light output centered near 564 nm (λ_max = 564.0 nm, T_max = 43.5 %, Δλ_1/2 = 9.0 nm), was inserted to permit monochromatic observations. When we studied the cells on intraocular lenses in vivo, we focused the microscope on the anterior surface of intraocular lens. The findings were recorded both in white light (3000°K) and monochromatic light illumination. Ektachrome 400 and Konica 100 films were used for white light observation. Tri-X pan film was used for monochromatic light examination.

Cytological preparation

Light microscopy with hematoxylin-eosin staining. The intraocular lenses explanted from rabbit eyes were fixed in buffered formalin for more than three hours, soaked in a mixture of acetic acid and alcohol for two seconds, rinsed in 70% alcohol and then stained with hematoxylin for 5 minutes. After the hematoxylin staining, the implants were rinsed in water for 10 seconds, stained with eosin for one minute following a graded series of alcohol for dehydration. After soaking in xylol for 30 seconds, the implants were embedded in Canada Balsam. The cells observed by specular microscopy were identified using an Olympus BH2 microscope.

Phase contrast and scanning electron microscopy. The intraocular lenses explanted from rabbit eyes were fixed in a combination of 2.5% glutaraldehyde and 5% formalin in 0.1 M phosphate buffer. The cells on the explanted intraocular lenses, soaked in the pool of fixative solution, were observed by phase contrast microscopy (Nikon Diaphot-TMD). The specimens were then processed for SEM. The explanted intraocular lenses were dehydrated by a series of graded alcohol and

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Figure 1. Schematic representation of specular microscopy to study the cells on intraocular lenses in rabbit eyes. Part of the incident light projected through the lower slit of the microscope is reflected at interfaces. The reflected light is collected through the upper slit of the microscope to make images. Aqueous humor, cells, and intraocular lens surface are of major importance as sources of reflection when we study the cells on intraocular lenses.

critical-point-dried using carbon dioxide as the transitional fluid. Dried specimens were mounted on specimen stubs, sputter-coated with gold, and examined in a JEOL JSM-T300 SEM operating at 10 kV.

Human subjects
The patients had implantation of posterior chamber intraocular lenses. One hour before specular microscopy, cycloplegic mydriatic (Mydrin P®) was prescribed every 30 minutes. Specular microscopy was performed in the same manner as in the animal experiments. All the examinations were conducted after the informed consents were obtained from the patients.

Results
Animal Experiments
Specular microscopy. The living cells observed on implanted intraocular lenses by specular microscopy are small round cells, dendritic cells with branching processes (fibroblast-like cells), oval or polygonal cells with relatively large cytoplasm, and giant cells (Figures 2a, 3a and 4a) which often spread more than 400 µm in diameter. The cell population on intraocular lenses is different in each eye; small round cells are commonly seen within two weeks after the intraocular lens implantation, while giant cells increase in number two weeks after surgery. In most cases, numerous cells tend to cluster around manipulation holes. These cellular components are demonstrated in interference images and sometimes show a shadow-cast appearance (Figures 2a, 3a and 4a). Figure 3a shows the interference images of uniform gray color (arrowhead) and Figure 4a demonstrates fringes of various interference colors, which are mainly recognized on cells with relatively large cytoplasm and giant cells. In contrast, small round cells and fibroblast-like cells are sometimes recognized in shadow-cast appearances (Figures 2a and 4a), which are very similar to the images obtained by differential interference microscopy. In addition, small round images of shadow-cast appearances are also noted in the center part of some giant cells (Figure 2a, arrowhead). However, shadow-cast images are generally more extensive on small round cells and fibroblast-like cells than giant cells (Figure 4a). Both the interference and shadow-cast images disappear by changing the angle of the incident light of the microscope; with only the cell outline and the pigment granules left on its images.

Light microscopy. The cellular components on explanted intraocular lenses (Figure 2b) are demonstrated by the histological examination with hematoxylin-eosin staining. The comparison of the images of the same cells between specular microscopy and the histological examination (Figures 2a and 2b) shows that giant cells recognized in vivo by specular microscopy are multinucleated cells while cells with long branching processes are mononuclear ones. More than one hundred nuclei are noted in a ring-like fashion in the center part of some giant cells and polymorphonuclear leukocytes are scattered over the intraocular lenses in some cases. All the cells are located on a thin membranous coating covering the implants.

Figures 2a and 2b also demonstrate that the images found using light microscopy, with hematoxylin-eosin staining, correspond, in general outline, with the shape of the giant cell in specular microscopic images. However, there is a difference in the exact outline of the lamella in the two figures, which is probably caused by the active movement of lamella during the time required to explant and fix the specimen. Although the nuclear membrane of giant cells are not identified by specular microscopy, small round images emerge in shadow-cast appearances (Figure 2a, arrowhead), which are located in the center of the giant cells, partly consistent with the
nuclei of the giant cells.

**Scanning electron microscopy.** The main cellular elements on the implants are histiocytes and giant cells. These cells are scattered on a thin membranous coating covering the intraocular lens surface. The morphological correlations of the same giant cells between the specular microscopy, phase contrast microscopy and SEM are shown in Figures 3a, 3b, 3c and 3d. It is demonstrated that, although the detailed shape of the lamella has changed, the general outline of the giant cell by phase contrast microscopy is consistent with that recognized by specular microscopy and SEM. Figures 4a and 4b also show the images of the same giant cell obtained by specular microscopy and SEM, respectively. Filamentous extensions spread from the edge of the flattened giant cell toward the surface of the intraocular lens (Figure 4b), but they are absent in the specular microscopic images (Figure 4a). Note that the small round cells recognized in vivo (Figure 3a) fell from the intraocular lens during the specimen preparation (Figure 3b), which was probably caused by the lower adhesion of the small cells compared to the giant cells.

**Human subjects**

The cells recognized on intraocular lenses in living human eyes are small round cells, cells with branching processes (fibroblast-like cells), round or polygonal cells and giant cells. Figures 5, 6 and 7 show interference fringes on some giant cells. These fringes are generated over the cells except on the pigment granules located on the center of the cytoplasm. Time lapse study of a giant cell in monochromatic light observation (Figures 5a and 5b) demonstrates that these concentric dark and light fringes change with the configuration of the lamella. In white light examination, interference colors on giant cells (Figure 6) change from light brown through purple, blue, white, yellow and red to distinct green toward the presumably thicker region of the cytoplasm. The series of interference colors toward the presumably thicker region is well correlated with those of interference color chart (Nikon) that is used to calculate the optical thickness of the specimen by differential interference contrast microscopy. In addition to the concentric fringes, a uniform gray interference area, which spread beneath the concentric color fringes (Figure 7), was noted on many giant cells. This gray area is located consistently within the outline of the cells and is readily recognized in the center part of the cell cytoplasm. Its size varies in each cell and it often occupies a broad area within the outline of the cells. These interference images are only generated on the cells with a relatively large cytoplasm and on giant cells. The difference between the concentric fringes and the gray interference is in the cell movement of well spread giant cells. That is, the time lapse study demonstrates that the concentric fringes change their configuration with the active movement of the lamella (Figure 5). The gray interference area, in contrast, does not show a direct relation with the movement of lamella. Both the concentric fringes

**Figure 2a.** Specular microphotograph showing living cells on intraocular lenses implanted in a rabbit eye three weeks after intraocular lens implantation. Fibroblast-like cells (F), cells with relatively large cytoplasm (H), and giant cells (G), were scattered over the intraocular lens surface. Small round images were recognized in the center part of the giant cell (Arrowhead). Small round cells and fibroblast-like cells were demonstrated in shadow-cast images. Arrow indicates the location of a manipulation hole. Bar = 100 µm.

**Figure 2b.** Light microscopy showing the same part as that of Figure 2a. The giant cell in Figure 2a turned out to be multinucleated cell. Approximately thirty minutes elapsed between specular microscopy and the fixation. Bar = 100 µm.

**Figure 3.** The same part of an intraocular lens implanted in a pigmented rabbit eye. **Figure 3a.** Specular photomicrograph showing living cells on intraocular lenses three weeks after intraocular lens implantation. Note small round cells (S), cells with processes (fibroblast-like cells, F) and a giant cell (G). Arrowhead indicates gray interference color in the center part of the giant cell. Bar = 100 µm. **Figure 3b.** The shape of the giant cell by phase contrast microscopy (Figure 3b) corresponded in general outline with that by specular microscopy (Figure 3a). Elapsed time between specular microscopy and the fixation was approximately thirty minutes. Most of the small round cells in Figure 3a fell from the implant. **Figures 3c and 3d.** Scanning electron micrographs showing part of the giant cell (arrowhead in Figure 3b) and lamella of the giant cell (arrow in Figure 3b) respectively. Bars = 10 µm.

and gray interference color disappear out by changing the angle of the incident light of specular microscopy.

**Interpretation of Interference Images**

Specular microscopy is based upon the optical principle of image formation by light waves reflected at interfaces of materials with different refractive indices. The image contrast depends on differences in reflectivity which originate from variations in refractive index differences across boundaries, that is different Fresnel reflectivity coefficients: \((n_1-n_2)/(n_1+n_2)\) (n: the refractive indices of media which make an interface). However, our results demonstrate that some images on intraocular lenses by specular microscopy depend not only on the difference of refractive indices of media but also on the interference phenomena of reflected light.

**Figure 8 shows probable ray path at interfaces when we study the cells on intraocular lenses by specular microscopy.** There are three important sources of reflection: the aqueous humor, cells, and the intraocular lens surface. Where the incident light encounters the aqueous humor-cell boundary (dorsal surface of the cell), part of the light is reflected and part is refracted at aqueous humor-cell interface (R1). The light refracted at the aqueous humor-cell interface travels through
the cell cytoplasm and is partly reflected at ventral surface of the cell facing an intraocular lens (R2). The time lapse study (Figure 5) demonstrates that the concentric dark and light fringes change with the configuration of lamella. This finding shows that these concentric fringes originate from the cell: the light reflected at the aqueous humor-cell interface and that reflected at the ventral surface of the cell recombine to interfere with each other.

Based on optical principles, interference fringes are generated in the optical condition:

\[ \Delta = (N + 1/2)\lambda, \text{ and } \Delta = N\lambda. \]  

where \( \Delta \) = optical path difference of lights, \( \lambda \) = wavelength of light, and \( N = 0, 1, 2, \ldots \) the order of interference.

Dark and light fringes are generated by monochromatic light, while the color fringes are generated by white light illumination, since white light includes beams of various wave lengths. The refractive index of the aqueous humor is 1.33366-1.33700 (Duke-Elder and Gloster, 1968) and that of polymethylmethacrylate intra-
ocular lens is 1.491. As the refractive index of living cells (Izzard and Lochner, 1976; Beck and Bereiter-Hahn, 1981) is higher than that of aqueous humor and lower than that of intraocular lenses, the incident light experiences a phase shift of $\pi$ at the aqueous humor-cell interface (dorsal surface of the cell) but no shift at the cell-aqueous humor interface (internal reflection). Then dark fringes are expected to be generated for $\Delta = NA$ and light fringes for $\Delta = (N + 1/2) \lambda$ in monochromatic light observation.

Two conditions of interference colors are also noted in white light illumination: One involves a phase
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**Figure 4.** The same giant cell on an intraocular lens implanted in a rabbit eye. **Figure 4a.** Specular photomicrograph showing a living giant cell on intraocular lenses in a rabbit eye four weeks after intraocular lens implantation. A series of interference colors and the gray interference color were generated on the giant cell. Small round cells were demonstrated in shadow-cast images. Bar = 100 µm. **Figure 4b.** Scanning electron micrograph showing the lamella of the giant cell in Figure 4a (Arrowhead). Filamentous extensions spread from the edge of the lamella toward the surface of the intraocular lens. Bar = 10 µm.

**Figures 5a and 5b.** Specular photomicrograph showing cell movement of a giant cell on an intraocular lens in a human eye. Elapsed time between Figures 5a and 5b was thirty minutes. The concentric dark and light fringes changed their configuration with the movement of lamella. Bar = 100 µm.

**Figure 6.** Specular photomicrograph showing a series of interference colors over a giant cell on an intraocular lens in a human eye, see text for explanation. Bar = 100 µm.

**Figure 7.** Specular photomicrograph showing complex interference images on a giant cell on an intraocular lens in a human eye. Gray interference color was recognized beneath the concentric color fringes. See text for further details and explanation. Bar = 100 µm.

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shift of \( \pi \) on reflection at one interface, while the other involves no phase shift. These two conditions are characterized by the colors produced by a thin plate in air in reflected light and those by a thin plate in air in transmitted light (Izzard and Lochner, 1976) and the difference of the two conditions is distinct in the first order and subsequent higher order fringes. A series of interference colors toward the presumably thicker region of the cells (Figure 6) is well correlated with interference color chart (Nikon) corresponding to the condition with phase shift of \( \pi \) and this finding supports the interpretation that the light reflected at the dorsal surface of the cell (R1) interferes with that at the ventral surface of the cell (R2).

Since the optical path difference (Figure 9) is expressed in the equation:

\[
\Delta = 2n d \cos \theta
\]  

(2)

where \( n \) = refractive index, \( d \) = the cell thickness, \( \theta \) = the angle of refraction, \( \cos \theta = 0.95 \pm 0.02 \) (15 < \( \theta \) < 20), the thickness of the cells between the neighbouring dark or light fringes is calculated as an approximation

\[
d = \left( \Delta / 2n \cos \theta \right) = 220 \text{ nm}
\]  

(3)

**Figure 8.** Probable ray path at interfaces when specular microscope is focused on the cells on intraocular lenses. Part of the incident light is reflected at aqueous humor-cell interface (R1; dorsal surface of the cell) and part is refracted. Part of the refracted light at aqueous humor-cell interface is then reflected at the ventral surface of the cell (R2); it will interfere with the light reflected at the aqueous humor-cell interface (R1). The light refracted at the ventral surface of the cell will be again reflected at the intraocular lens surface (R3) and interfere with the light reflected at the ventral surface of the cell (R2).

**Figure 9.** Schematic representation of ray paths at interfaces to calculate the cell thickness. \( i \) = angle of incidence. \( \theta \) = angle of refraction. \( d \) = cell thickness. See text for explanation.
(Izzard and Lochner, 1976). We calculate the cell thickness up to about 4 \( \mu m \) in monochromatic observation.

In addition to these interference fringes, our findings also demonstrate a uniform gray area (Figure 7). It indicates that there is another interface to make the gray interference. According to the interference color chart, the gray interference is predicted to be the zero order interference and is consistent with the presence of a narrow space between the cell and the intraocular lens, as diagrammed in Figure 8: The light refracted at the ventral surface of the cell will again be reflected at the surface of intraocular lens (R3) and interfere with the light reflected at the ventral surface of the cell (R2).

In summary, the optical principle of specular microscopy is basically the same as that of the interference reflection microscopy and the optical events on the giant cells are optically interpreted in the same manner as those by the interference reflection microscopy.

**Discussion**

As implanted intraocular lenses are settled in eyes for a long period, the cellular reaction on intraocular lenses may play a role in ocular pathology such as iritis, glaucoma and cystoid macula edema. This is the reason why many kinds of intraocular lenses made of different materials and with surface modifications such as silicon, hydrogel, etc., are coming into use to obtain better biocompatibility. The present study gives us helpful information to evaluate the cell-intraocular lens interaction in living human eyes.

To study the foreign body reaction on intraocular lenses, we implanted polymethylmethacrylate intraocular lenses in the anterior chamber of rabbit eyes. The observed findings on intraocular lenses were compared with other reports by light microscopy (Wolter, 1982, 1985; Uenoiyama et al., 1988), SEM (Kappelhof et al., 1986; Uenoiyama et al., 1988; Bryan III et al., 1985; Versura et al., 1987). However, those observations performed independently by light microscopy or SEM, included artifacts which occur during the specimen preparations (Stacholy et al., 1989). In our work, the combined method of specular microscopy, a biomicroscopy with light microscopy and SEM, allows recognition and elimination of the specimen preparation artifacts.

In the present study, specular microscopy was applied as an interference reflection microscope. However, conversely interference reflection microscopes currently available cannot be used to study the cellular reaction on intraocular lenses, because the working distance of the interference reflection microscope is too short to see the cells on the surface of intraocular lenses.

In cell biology, the interference reflection microscope has been widely used to investigate cell-substrate interaction in tissue culture condition. Curtis (1964) first introduced this technique to study the structure of the adhesion of cultured cells to substrate and its optical principle has been discussed by many investigators (Curtis, 1964; Izzard and Lochner, 1976; Bereiter-Hahn et al., 1979; Gingell, 1981; Bereiter-Hahn, 1985; Verschueren, 1985). Izzard and Lochner (1976) pointed out the effect of angle of the incident light demonstrating that with the higher illuminating numerical aperture, the higher order interference fringes are restricted. As the illuminating numerical aperture of specular microscope is 0.3, the generation of higher interference fringes by specular microscopy agree with the results obtained by interference reflection microscopy of low illuminating numerical aperture (Curtis, 1964; Izzard and Lochner, 1976; Bereiter-Hahn, 1985).

The adhesions of cells to substrate in various cell types were also studied by many researchers (Curtis, 1964; Izzard and Lochner, 1976; Abercrombie et al., 1977; Bereiter-Hahn et al., 1979; Gingell, 1981; Heath, 1982). Ploem (1975) studied the attachment of macrophages to substrate in culture by interference reflection microscopy and reported that there are dark zones close to the cell boundary. This dark area was interpreted to originate from the thin lamella of cells by Gingell (1981) and Heath (1982). Abercrombie et al. (1977) also investigated the adhesion of cultured macrophages to substrate using interference reflection microscope and reported that there are only gray areas beneath the polymorphs and the macrophages. His findings probably correspond to the broad gray interference area beneath concentric fringes in this study (Figure 7). This gray area is accepted as the expression of the separation between cells and a substrate in culture (Izzard and Lochner, 1976; Abercrombie et al., 1977; Verschueren 1985).

In addition to the interference images, the present study demonstrates shadow-cast appearances of fibroblast-like cells, small round cells and small round images in the center part of the giant cells (Figures 2a, 3a, and 4b). These findings were very similar to the "relief mode" noted on corneal endothelium by specular microscopy (Sherrard and Buckley, 1982). Kachar (1985) demonstrated that the optical shadowing was obtained by oblique light illumination and the shadow-cast images recognized by specular microscopy may originate from the diffraction of the light obliquely reflected at interfaces.

In conclusion, the complex images of concentric fringes and the gray interference color on giant cells, recognized by specular microscopy were interpreted to be caused by interference phenomena, arising from both the cell cytoplasm and the separation between the cell and the intraocular lens, respectively. Shadow-cast images were also noted. Specular microscopy can be applied as an interference reflection microscopy to study cell-intraocular lens interaction in living human eyes.

**Acknowledgement**

We thank Professor C.S. Izzard and Mr. H Takenaka for their helpful suggestions on the image interpretations. We also acknowledge Mr. Y. Yamada and Mr. Y. Ishii for their valuable contribution to SEM.
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observations. Intraocular lenses were supplied by Menicon Company, Japan, and Healon was provided by Pharmacia, Japan, for animal experiments.

References


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Discussion with reviewers

P. Versura: Do the authors have any idea on the real accuracy by which a degree of the gray color can account for the distance between the cells and the implant?

Authors: In the present set-up of our system, it is very difficult to directly determine the exact hue of gray interference color and calculate the cell-intraocular lens separation, because the numerical aperture of our specular microscope corresponds to 0.3 (low illuminating numerical aperture) and the cell body will have considerable effect on its measurement as discussed by Izzard and Lochner (1976). So, at present time, we do not have a proper method.

Reviewer III: Filamentous extensions did not appear in the specular microscopic image. The reason could be that the "filamentous extensions" were not present in the live cell. In fact, the structures look more like retraction fibers, and could have been produced by withdrawal of the cell in response to removal of the lens from the eye and/or fixation. This should be clarified. Can the authors fix the "lens" in situ by perfusion and then remove the lens?

Authors: We are convinced that the "filamentous extensions" could be caused by the fixation procedure. We can fix intraocular lenses in situ by irrigating the anterior chamber with fixative solution prior to explantation. After this procedure we can observe the fixed cells again and compare the findings of the same cells before and after the fixation. This attempt is ongoing, we will present these results in a future paper.

P. Versura: The authors state that the round cells are typical of the first two weeks postoperatively. On Figure 3b these cells disappear during the preparation of the specimens for the phase contrast microscopy, which would indicate a less adhesion of these small cells as compared to the adhesion displayed by the giant cells. Can the authors comment on the nature of these small round cells and their degree of stickiness onto the intraocular lens surfaces?

Authors: These small round cells were probably in the process of adhesion to the intraocular lens and had not come to the stage of spreading when we explanted the intraocular lens. In contrast, the giant cell had a well spread contour (Figures 3c and 3d). This may contribute to the results.

P. Versura: The so called "surface factors" of the prosthesis (holes, irregularities, scratches, etc.) are believed to be an important issue for adhesion mechanisms in that they represent an easy point of attach for cells and proteins. Have the authors observed specific concentrations of cells in correspondence of these particular points?

Authors: The cells on intraocular lenses tended to cluster around manipulation holes in rabbit eyes. We have studied more than five hundreds cases in humans and sometimes found a few scratches which were made during the cataract surgery. However, there was no tendency of cells to accumulate around these scratches.

P. Versura: The authors should have noted different populations of cells onto the intraocular lenses inserted in the human eyes, in relation to the clinical course of the implant. Have they attempted to quantify the cells in total and the relative number of the round / fibroblast-like / oval / giant cells over the time and in presence of eventual, even light, post-operative complications?

Authors: Yes, we have. The relative number of giant cells, generally, increases with the time course. In addition, the giant cell formation is extensive in some cases. There may be some factors to promote the formation of giant cells.