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Original

Differences in Intraocular Lens Surface Properties and Lens Epithelial Cell Behavior

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Summary

Posterior capsule opacification is a common complication following cataract surgery. However, its incidence varies depending on the type of intraocular lens (IOL) used. In this study, we analyzed the adhesion and movement of lens epithelial cells (LECs) in the early stages after cataract surgery by continuously observing LECs seeded on the surfaces of different types of IOLs. PY-60AD and XY1 hydrophobic acrylic IOLs were used. Each posterior surface was modified to increase the adhesion. The IOLs were placed in culture dishes with an exposed posterior or anterior surface (three each). LECs extracted from white rabbit lenses by trypsinization were seeded onto IOLs. LEC movement and shape were recorded by microscopy using a cell culture observation system after 6 h of contact between LECs and IOLs. The cumulative distance traveled was calculated from the LEC trajectories after comparing the shapes of the LECs. We observed that LECs were more likely to adhere to XY1 modified posterior surface. The speed of LEC movement decreased after adhesion. These findings support the conclusion that increasing the adhesiveness of IOL surfaces inhibits the LEC movement.

Key Words: cell behavior, cell culture, lens epithelial cells, secondary cataract, surface modification

Introduction

Late-onset posterior capsule opacification (PCO) is a well-known complication after cataract surgery. The reported incidences were 11.8%, 20.7%, and 28.4% at one, three, and five years after surgery, respectively.[1,2] Efforts to reduce late-onset PCO have been made, and current intraocular lenses (IOLs) control invasion of lens epithelial cells (LECs) into the posterior capsule by using a square-edge design around the optic, which has reduced late-onset PCO.[3-5] However, even with square-edge designs, late-onset PCO develops in many cases after a long period of time, eventually requiring posterior capsulotomy using a neodymium-doped yttrium aluminum garnet (Nd:YAG) laser.[6]

To further reduce late-onset PCOs, we developed a method to modify the surfaces of IOLs to increase cell adhesion.[7,8] The method has been reported to be clinically effective.[9,10] However, there are many unknowns regarding the behavior of LECs in lens capsules. To our knowledge there have been no reports on this topic. In this study, we analyzed the adhesion and movement of LECs in the early stages after cataract surgery by experimentally and continuously observing

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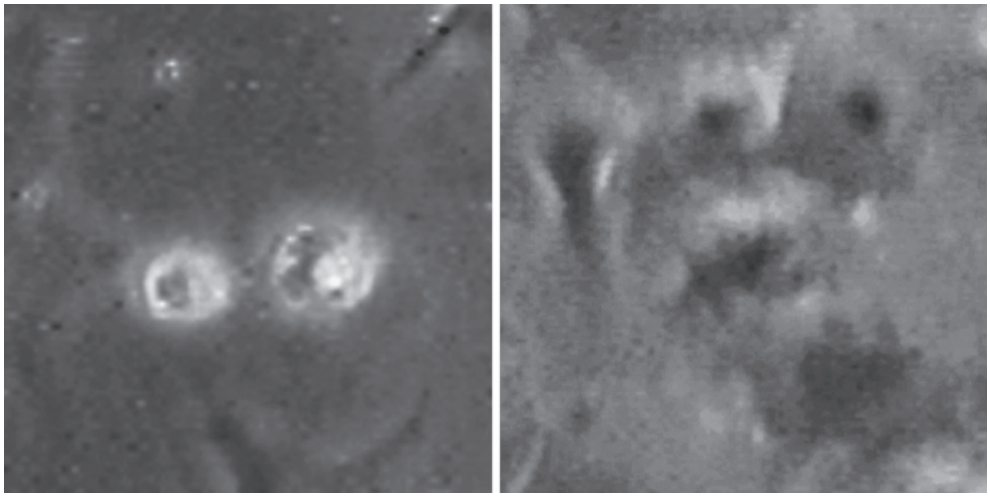

Figure 1 Changes in lens epithelial cells (LECs) after seeding on intraocular lens.
A: LECs seeded on IOLs were initially observed as round, non-adherent cells.
B: As the LECs adhered to the IOL over time, the border became indistinct due to the extension of focal adhesions.

LECs seeded on the surfaces of IOLs.

Materials and Methods

LEC adjustment

Eyeballs from 8-week-old white rabbits were enucleated, and the cornea and iris were separated to extract lens capsules. Each extracted lens capsule was desquamated and treated with a trypsin-EDTA solution (Invitrogen, Carlsbad, CA, USA) at 37℃ for 10 min. The dissociated LECs were then cultured for one week at 37℃ with 5% CO_2 in a minimum essential medium (MEM) containing 10% fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS, USA). The obtained LECs were subcultured, and cells that had been passaged three-five times were used for the experiment. The LECs were adjusted to a concentration of 20×10^4 cells in the delivered volume before being seeded onto IOLs.

Preparation of IOLs and seeding of LECs

A hydrophobic acrylic IOL material, PY-60AD, and XY1 (Both from HOYA Surgical Optics, Tokyo, Japan), a next-generation hydrophobic acrylic IOL material, were prepared as follows: The posterior surface of each XY1 sample was modified by ultraviolet/ozone treatment to reduce late-onset PCO and increase cell adhesion. A 35-mm culture dish was prepared, and six IOLs were added: three with the posterior surface exposed and three with the anterior surface exposed. Following the seeding of the modified LECs at a volume of 2 mL onto each IOL surface, MEM containing 10% FBS was added. The culture dish was placed in the cell culture observation system of a model IX70 microscope (Olympus, Tokyo, Japan) at 37℃ and 5% CO_2. LECs were recorded continuously for 6 h after contact with the IOL using a Video Field Recorder (Roland, Shizuoka, Japan). The obtained video data were compressed to 20 s and edited to allow short-term observation of the state of the cells.

Analytical method for LEC after seeding

Images were acquired two, four, and six h after recording began, and LEC shapes were compared (Fig. 1). When LECs first came into contact with the IOL, they were observed as round, non-adherent cells. As the LECs adhered to the IOL over time, the border became indistinct because of the extension of focal adhesions from the LECs. Cells were counted within an arbitrary 100 μm^2 of each IOL surface, and the percentage of LECs adhering to the IOL was calculated. In addition, to analyze the movement of LECs on the IOL, the nuclei of adherent LECs were marked at the time of cell contact. The six-hour video was divided into 600 still images and 20 randomly selected cell nuclei were

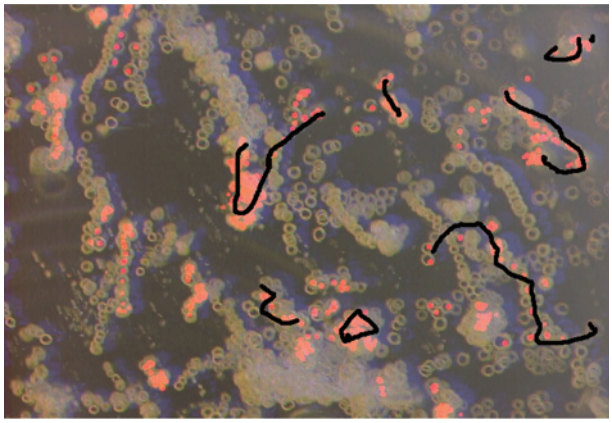

Figure 2 Measurement of lens epithelial cell (LEC) movement distance.
After marking 20 randomly selected LEC nuclei recorded on video for 6 hours after LEC seeding, the data was divided into 600 still images. The images were multiplexed using image analysis software, and the trajectories of the marked cell nuclei were mapped to calculate the movement distance.

marked. These images were multiplexed using StarStax® (Esslingen, Germany) image analysis software and the trajectories of the marked cell nuclei were mapped to calculate the cumulative distance (CD value) (Fig. 2). The Tukey-Kramer test was used for statistical analysis.

Results

Changes in LECs after seeding on PY-60AD and XY1 IOLs

In PY-60AD, some LEC focal adhesions extended and transformed into adherent cells after two h on both the anterior and posterior surfaces of the optics. The number of adherent cells increased with time. However, on the XY1 anterior surface, cell adhesion was difficult, and many non-adherent LECs were observed, even after six h. On the XY1 posterior surface, which had been surface-modified, LEC focal adhesions extended immediately upon contact with the IOLs, and the number of adherent cells increased (Fig. 3). Fig. 4 shows the percentage of adherent LECs in each group. On both the anterior and posterior surfaces of PY-60AD, the number of adherent LECs increased over time, with 33.0% on the anterior surface and 31.25% on the posterior surface six h after seeding. On the XY1 anterior surface, the percentage of adherent LECs was 18.0% even after six h; whereas on the posterior surface, LEC adhesion was observed after two h and increased to 49.4% after six h.

Analysis of LEC movement speed

When the LEC movement distance was analyzed six hours after adhesion on PY-60AD, it was 9.2 ± 2.6 μm on the anterior surface and 12.5 ± 3.7 μm on the posterior surface of the optics (Fig. 5). Although only a small number of LECs adhered to the XY1 anterior surface, the determined CD value was as high as 18.1 ± 5.8 μm. In the modified posterior surface, to which many LECs adhered, the CD value was the shortest at 4.9 ± 1.0 μm. Comparison of the CD values of the anterior and posterior surfaces of XY1 revealed a statistically significant difference (Tukey-Kramer, $p < 0.01$).

Discussion

Although PCO and anterior capsule contraction after cataract surgery have been demonstrated histologically,[11] few studies have focused on the cell behavior of individual LECs. In this *in vitro* experiment, LEC behavior varied depending on IOL type and properties. LECs easily adhered to highly adhesive IOLs, and their movement speed decreased after adhesion.

PCO is a common complication after cataract surgery[1] and can cause visual function decline as it progresses, such as decreased contrast sensitivity.[12] Various studies have sought to prevent PCO, and one study states that a square edge around the optic of the IOL bends the capsule, which can inhibit PCO.[3-5] However, PCO can still progress efficiently over a prolonged period and may require treatment using Nd:YAG laser posterior capsulotomy.[6] Other methods

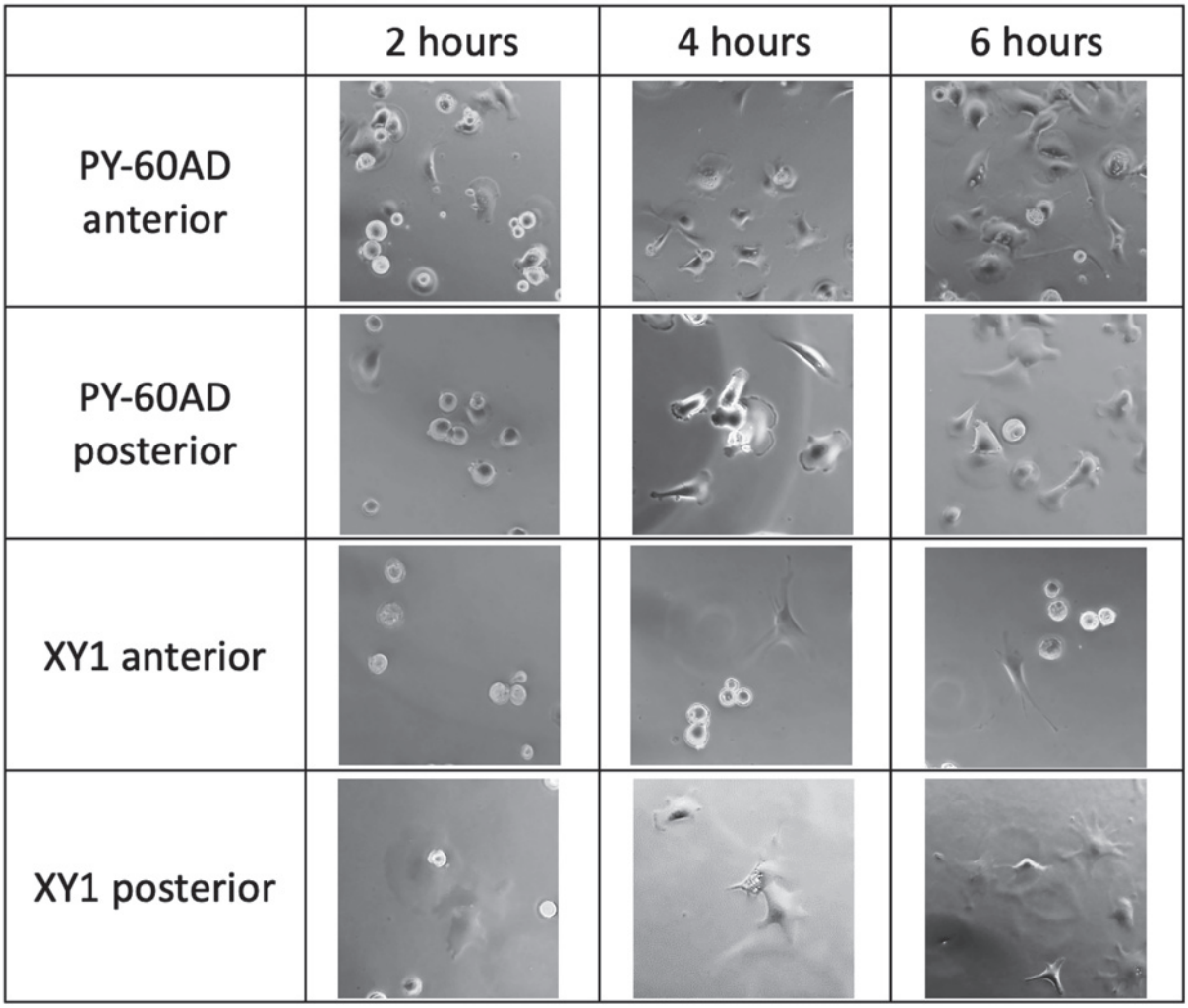

Figure 3 Changes in lens epithelial cells (LECs) after seeding on various intraocular lenses.
On both the anterior and posterior surfaces of PY-60AD, the number of adherent LECs increased over time once LECs were seeded. On the anterior surface of XY1, there were many non-adhered LECs, even after time had passed. In contrast, on the posterior surface of XY1, LEC adhesions had progressed after 4 hours.

besides square-edge design may be necessary for the long-term reduction of PCOs.

To reduce PCO using a square-edge design, we devised a method to increase cell adhesion to the IOL surface.[7,8] Ultraviolet/ozone treatment can physically and chemically modify the surfaces of various materials, improving their wettability and adhesion. During this treatment, active species, such as reactive oxygen species and ozone, modify the material surface, generating -OH and -COOH groups to improve wettability and adhesion.[13] A clear reduction in PCO was observed in animal experiments.[7,8] Experiments have proved that modifying the IOL surface between the IOL and posterior capsule using adhesive proteins, such as LEC and fibronectin, is effective in reducing PCO. Clinical reports have also shown that the surface modification of XY1 is effective in reducing PCO.[9,10]

During cataract surgery, an IOL was inserted into the lens capsule. Experiments in rabbits undergoing cataract surgery revealed that LECs covered the peripheral part of the anterior capsule, equator, and central part of the posterior capsule in the early postoperative period.[14] The same is expected to occur in the human lens.[15] Therefore, LECs come into contact with the IOL immediately after the surgery. Investigating the relationship between IOL material and LECs may be useful in considering the mechanism for reducing PCO. LEC adhesiveness varies greatly, depending on the IOL material used.[16] Increasing adhesiveness reduces PCO; however, only a few studies have exam-

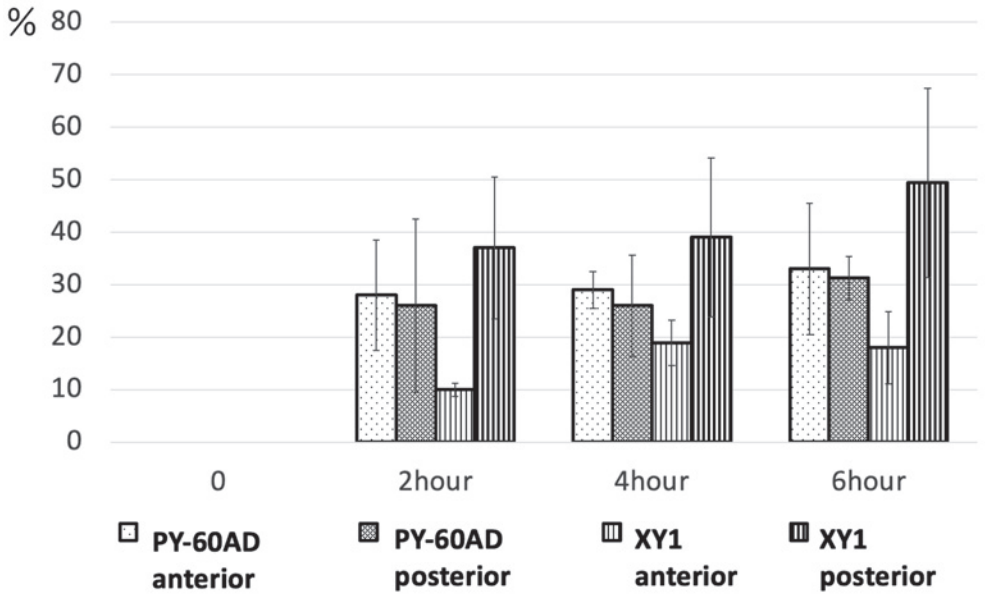

Figure 4 Percentage of adherent lens epithelial cells (LECs) after seeding on various intraocular lenses (IOLs).
On both the anterior and posterior surfaces of PY-60AD, the number of adherent LECs increased over time to approximately 30% following seeding. On the anterior surface of XY1, 18.0% of LECs adhered, even after six hours. On the posterior surface of XY1, adherent LECs were observed after two hours, and increased to 49.4% after six hours.

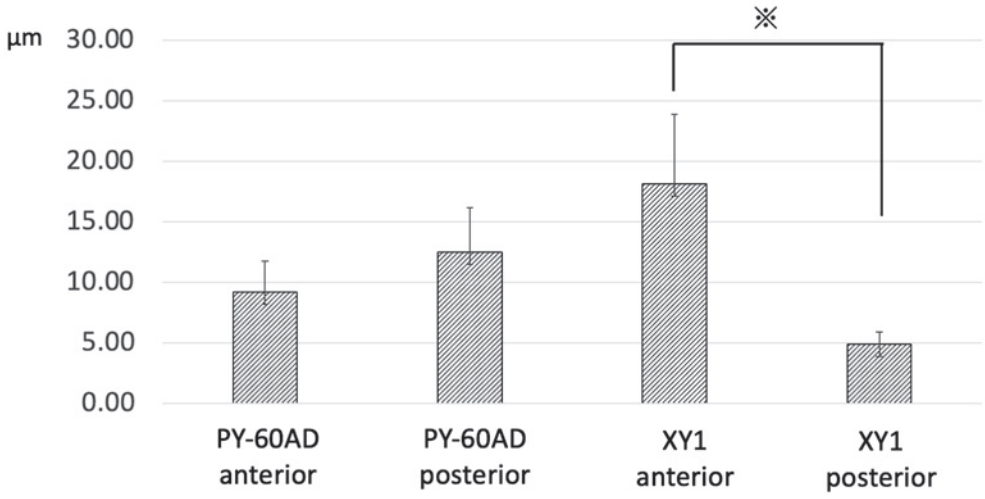

Figure 5 Lens epithelial cell (LEC) movement distance after seeding on various intraocular lenses (IOLs).
The overall moving distance on both the anterior and posterior surfaces of PY-60AD was approximately 10 μm. Cell movement was statistically significantly greater on the anterior surface of XY1 compared to the posterior surface (18.1 ± 5.8 vs. 4.9 ± 1.0 μm; *Tukey-Kremer $P < 0.01$).

ined IOL materials and LEC behavior.

This experiment showed that increasing the adhesiveness of the IOL surface inhibited the behavior of adhered LECs. These findings suggest that decreased cell movement may be one of the mechanisms responsible for reducing PCO. However, the observation period in this study was only six hours, and it is unclear how decreased cell movement impacts the reduction of PCO. Further research is required to investigate the reduction in PCO, including long-term cell behavior (adhesion and movement) studies.

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