Contact inhibition of migrating lens epithelial cells at the capsular bend created by a sharp-edged intraocular lens after cataract surgery

Okihiro Nishi, MD, Naoki Yamamoto, PhD, Kayo Nishi, MD, Yutaro Nishi, MD

PURPOSE: To investigate whether the lens epithelial cells (LECs) at the capsular bend created by a sharp-edged intraocular lens (IOL) are in the G_0 phase of the cell cycle.

SETTING: Nishi Eye Hospital, Osaka, Japan.

METHOD: A CeeOn Edge silicone IOL (AMO) with sharp edges was implanted in 1 eye and a Phaco-Flex II silicone IOL (AMO) with rounded edges in the contralateral eye after standard cataract surgery in 6 rabbits. Immunohistochemical staining for the Ki-67 antibody was performed 1 day, 3, 4, and 7 weeks after surgery.

RESULTS: In eyes with the sharp-edged IOL, LECs with thin, elongated nuclei accumulated at, but did not extend beyond, the capsular bend and stained negative for the Ki-67 antibody, indicating that they were in the G_0 phase of the cell cycle. In contrast, in the eye with the round-edged IOL, continuous migration of a predominantly monolayer of LECs over the IOL and onto the posterior capsule occurred. These cells were Ki-67 positive, indicating that they were proliferating.

CONCLUSIONS: Lens epithelial cells at the capsular bend of sharp-edged IOLs were in the G_0 phase of the cell cycle, indicating that they were contact inhibited. These findings support the theory the sharp posterior optic edge of the IOL inhibits LEC migration, reducing formation of posterior capsule opacification. Whether these LECs can reactivate when the capsular bend is eliminated by later formation of a Soemmerring's ring requires further studies.

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Experimental[1–4] and clinical studies[5–13] show that the capsular bend formed by the sharp posterior optic edge of an intraocular lens (IOL) inhibits lens epithelial cell (LEC) migration onto the posterior capsule, reducing posterior capsule opacification (PCO). We postulated that contact inhibition of migrating LECs is induced at the capsular bend.[1–3] Our theory is based on the observation that cells in the rectangular well bottom of a cell culture dish tend to form layers when becoming confluent but cannot climb the vertical wall of the culture dish. The cell cycle consists of the G_1, S, G_2, M, and G_0 phases. When cells are in the G_0 phase, they are in a state of rest; that is, they are not proliferating. Accordingly, we postulated that the migrating LECs at the capsular bend should also be in the G_0 phase.

To test our hypothesis, we investigated whether LECs at the capsular bend were influenced by contact inhibition, and, if so, were in G_0, the nonproliferating phase.

MATERIALS AND METHODS

Surgical Procedure

This experimental study used rabbits. All animal procedures were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

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From the Nishi Eye Hospital (O. Nishi, K. Nishi), Osaka, and the Fujita Health University Joint Research Laboratory (Yamamoto), Toyoake, Japan; Universitäts-Augenklinik (Y. Nishi), Heidelberg, Germany.

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Corresponding author: Okihiro Nishi, MD, Nishi Eye Hospital, 4-14-26 Nakamichi, Higashinari-ku, Osaka 537-0025, Japan. E-mail: okihiro@nishi-ganka.or.jp.

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Six Dutch or albino rabbits weighing 1.5 to 2.0 kg had implantation of a PhacoFlex II SI-40NB silicone IOL (AMO) with rounded edges in 1 eye and CeeOn Edge silicone IOL with sharp edges (AMO) in the contralateral eye. All surgeries were performed with the animals under anesthesia. The same surgeon (O.N.) performed standard phacoemulsification and aspiration and IOL implantation as previously reported.[1]

Immunohistochemical Examinations

One day (1 rabbit), 3 weeks (2 rabbits), 4 weeks (2 rabbits), and 7 weeks (1 rabbit) after surgery, immunohistochemical examinations of each eye were performed using the following protocol: After the rabbit was killed by an intravenous injection of ketamine sulfate (0.3 mg/kg) and succinylcholine chloride (0.15 mg/kg), the eyes were enucleated, and a small incision was made at the limbus and equatorial sclera to facilitate the uptake of the tissue-fixation solution. The specimen was immediately placed in a fixation solution (Yamamoto's Rapid Fix Solution) for several days and then embedded in paraffin. Briefly, this solution is neutrally buffered, with a low concentration of a protein cross-linker. The specimen was sliced with a microtome and put on a slide glass to permit Ki-67 and proliferating nuclear antigen (PCNA) staining.

Ki-67 Staining

Ki-67 is a nucleus protein that is detectable in all phases of the cell cycle except the G_0 phase.[14,15] Paraffin sections (5 μm) were incubated with anti–Ki-67 rabbit polyclonal antibody (1:50, Abcam Ltd.) for 30 minutes at 37°C. Bound antibody was detected with the Histofine Simple Stain MAX-PO(R) reagent using diaminobenzidine tetrahydrochloride as the substrate, and the sections were counterstained with Mayer's hematoxylin.

Proliferating Nuclear Antigen Staining

This nucleus protein is detectable in all phases of the cell cycle except the G_0 phase. However, in the beginning phase of G_0, it is not negative and is still positive. This staining usually serves as a control of Ki-67 staining. Paraffin sections (5 μm) were incubated with anti-PCNA mouse monoclonal antibody (1:100, Ylem S.R.L.) for 30 minutes at 37°C. Bound antibody was detected with the Histofine Simple Stain MAX-PO(M) reagent using diaminobenzidine tetrahydrochloride as the substrate, and the sections were counterstained with Mayer's hematoxylin.

RESULTS

Table 1 summarizes the histopathological findings.

Comparison Between Ki-67 and Proliferating Nuclear Antigen Staining

In general, slightly more positive cells were observed with PCNA staining in all specimens, which may verify that PCNA staining served as a control for Ki-67 staining (Figure 1).

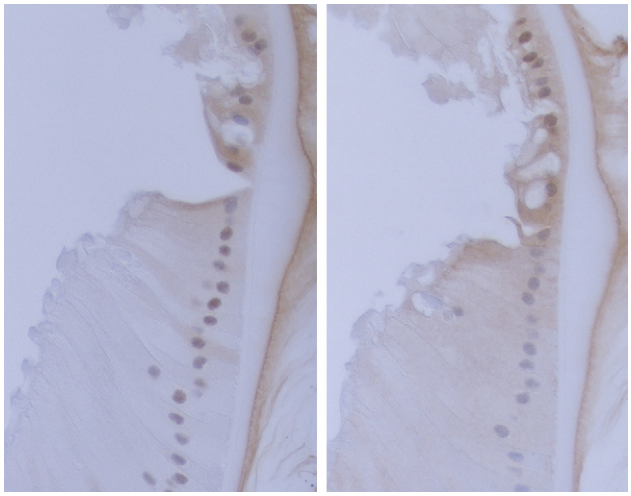

Figure 1. Comparison between PCNA and Ki-67 staining. Lens epithelial cells beneath the anterior capsule at the preequatorial region 4 weeks after surgery in the eye that received a PhacoFlex II IOL with round edges. *Left*: The PCNA staining served as a control. Note that the lens fiber cells (elongated cells) are still positive, with decreasing intensity of the brown nucleus staining in the consecutive row. *Right*: Ki-67 staining. Note that the lens fiber cells are all Ki-67 negative. This is because PCNA staining is still positive at the beginning of the G_0 phase.

Capsules in Eyes with Sharp-Edged Intraocular Lenses

One day after surgery in eyes with the sharp-edged CeeOn IOL, the anterior subcapsular LEC layer contained many cells, the nuclei of which stained positive for Ki-67. Migration of LECs from the preequatorial germinative zone was not observed. No capsular bend formation was noted.

Three, 4, and 7 weeks after surgery, a sharp capsular bend was observed in all specimens (R2, R4, R5, and R6 in Table 1, Figure 1 (left)). Many anterior subcapsular LECs showed Ki-67 positive nuclei. Lens fiber cells were seen on the posterior capsule, and their nuclei were all Ki-67 negative.

Beneath the anterior capsule and lens fiber cells on the posterior capsule was an accumulation of LECs, some of which were Ki-67 positive. This accumulation of LECs tapered toward the capsular bend between the side edge of the IOL optic and posterior capsule, forming a narrow triangle. With the exception of 1 or 2 cells that were not adjacent to the capsular bend but far from it, all nuclei of these LECs were Ki-67 negative, indicating that they were in the G_0 phase of the cell cycle (Figure 2, *B*).

At the sharp capsular bend, no LECs were found. Behind the bend, a small amount of migrating LECs were observed on the posterior capsule–forming layers, (Figure 2, C) except in rabbit R5, in which no

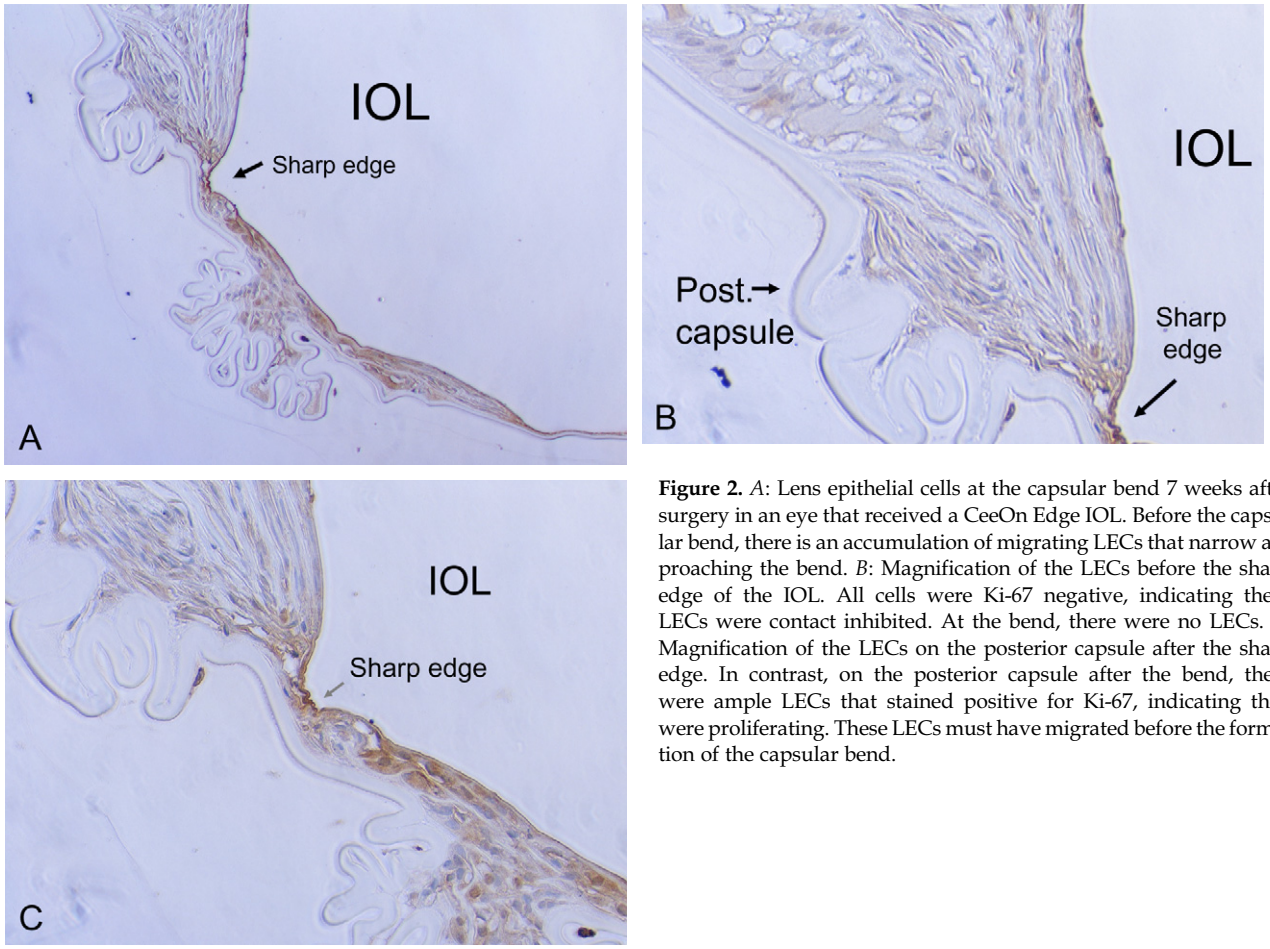

Figure 2. *A*: Lens epithelial cells at the capsular bend 7 weeks after surgery in an eye that received a CeeOn Edge IOL. Before the capsular bend, there is an accumulation of migrating LECs that narrow approaching the bend. *B*: Magnification of the LECs before the sharp edge of the IOL. All cells were Ki-67 negative, indicating these LECs were contact inhibited. At the bend, there were no LECs. *C*: Magnification of the LECs on the posterior capsule after the sharp edge. In contrast, on the posterior capsule after the bend, there were ample LECs that stained positive for Ki-67, indicating they were proliferating. These LECs must have migrated before the formation of the capsular bend.

LEC migration onto the posterior capsule was observed. The posterior capsule there showed complicated folds. These LECs diminished slowly, forming a monolayer, and disappeared centrally. The cells were extremely thin and elongated. Many of the LECs behind the capsular bend showed Ki-67 positive nuclei indicating that they were proliferating (Figure 2, *A* to *C*).

In summary, the typical common finding at the capsular bend or sharp edge was an accumulation of LECs whose nuclei were mostly thin, elongated, and Ki-67 negative, indicating the G_0 phase. At the equatorial capsule, lens fiber cells (Soemmerring's ring) were seen, often in abundance. The Ki-67 negative cells that accumulated at the sharp edge appeared to halt the migration of the lens fiber cells. Beyond the sharp edge site and toward the posterior capsule, some LECs were seen whose nuclei were round and not thin or elongated; that is, they had a normal appearance and were Ki-67 positive indicating that they were proliferating. These LECs diminished in number approaching the central posterior capsule, at which point they disappeared.

Capsules in Eyes with Round-Edged Intraocular Lenses

No sharp capsular bend formation was seen in eyes with the round-edged PhacoFlex II IOL. Beyond the germinating LECs, occasional lens fiber cells were seen. Between these fiber cells and the LECs beneath the anterior capsule was an accumulation of LECs, which tapered toward the posterior optic edge area. These LEC layers became narrower but were not disrupted at the posterior optic edge. They were narrow at the posterior round optic edge but migrated continuously onto the posterior capsule, as opposed to the posterior capsule in eyes with the sharp-edged IOL, in which no cells were found at the bend (R2, R3, R5, and R6 in Table 1) (Figure 3). The nuclei of these LECs were round and not thin or elongated and were predominantly Ki-67 positive.

DISCUSSION

This study would have been more exact had the 2 IOLs differed only in the construction of their optic edge. Although theoretically ideal, this is not possible given the

Table 1. Accumulation of the Ki-67 negative LECs at the capsular bend.

			Right		Left	
Rabbit	Postop Period	IOL Edge	Accumulation of Ki-67 Negative LECs	Ki-67 Positive LECs on PC	Accumulation of Ki-67 Negative LECs	Ki-67 Positive LECs on PC
R1	1 d	Sharp	Many LECs beneath the anterior capsule stained Ki-67 positive, but no LEC migration onto PC			
		Round				
R2	3 wk	Sharp	NA	NA	+	+
		Round	NA	NA	–	++
R3	3 wk	Sharp	NA	NA	NA	NA
		Round	–	++	–	++
R4	4 wk	Sharp	+	–	+	+ (isolated)
		Round	–	–	None	None
R5	4 wk	Sharp	++	–	++	–
		Round	–	++	–	+++
R6	7 wk	Sharp	+++	+	+++	+
		Round	–	+++	–	+++

+ = few; ++ = moderate; +++ = abundant; LECs = lens epithelial cells; NA = not applicable; PC = posterior capsule; Right and Left = both sides of the capsular bag in a histological section

commercially available IOLs. Moreover, many experimental and clinical studies have shown that it is the edge design that is the crucial and decisive factor in the inhibition of LEC migration, regardless of IOL material or design. Therefore, we used 2 types of silicone IOLs, the edges of which were sharp or round, with the full awareness that there was some dissimilarity between the IOLs in the silicone's chemistry constituents and their design. As discussed in the study methodology, the specific histopathological findings in this study were interpreted and correlated with the edge design of the IOL optic alone.

Ki-67 is a protein in the cell nucleus and can always be observed in all phases of the cell cycle except the G_0 phase. Therefore, a cell-staining pattern negative for Ki-67 indicates the cell is in the G_0 phase and is not proliferating.[14,15]

In this study, we investigated the expression of the unique protein Ki-67 in the nuclei of LECs at the capsular bend in the eyes with a sharp-optic-edge IOL design. In these eyes, all LECs that accumulated in multiple layers along the side edges of the IOLs before the capsular bend were Ki-67 negative in their nuclei, with the exception of a few cells in some eyes, indicating that most LECs were in the G_0 phase. These findings stand in stark contrast to the Ki-67 positive LECs abundantly observed beyond the capsular bend toward the posterior capsule.

We postulate that contact inhibition of the LECs is induced by the posterior capsular bend. We have shown in vitro and in vivo[16,17] that contact inhibition of LECs can be induced at the capsular bend. In a rectangular well bottom in cell culture, LECs never climb up the well wall. The histopathological sections of animal eyes show that in eyes with a capsular bending ring, LECs at the ring form layers but never migrate centrally along the posterior capsule.[17] Lens epithelial cell migration has been occasionally observed clinically at the slitlamp in human eyes with a capsular bending ring. However, in these cases, the migration was a result of the discontinuity of the capsular bend. This phenomenon can occur when the eyelets of the capsular bending ring are widely separated, as

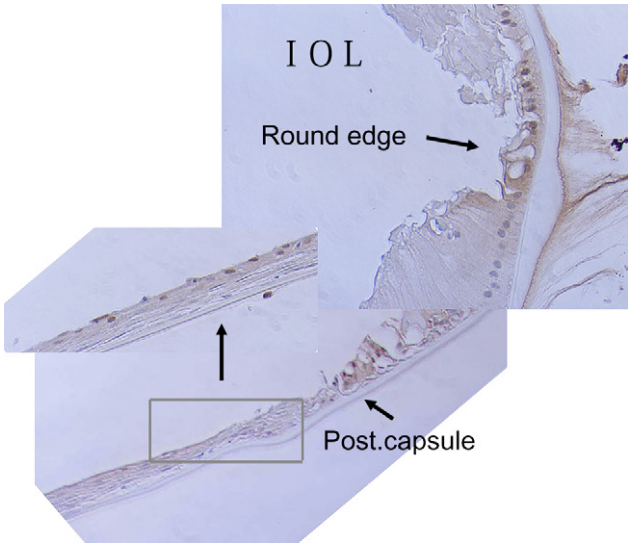

Figure 3. Lens epithelial cells at the capsular bend in an eye with a PhacoFlex II with rounded edges. The LECs showed continuous migration over the bend and contained Ki-67 positive nuclei, indicating that the LECs were not contact inhibited.

is the case with large-diameter capsular bags. However, LECs will be contact inhibited at a rectangular structural impasse. The results in the present experimental study are further evidence at a molecular biological level of our concept of contact inhibition. Migrating LECs were contact inhibited at the capsular bend created by the sharp optic edge of an IOL.

This contact inhibition theory is not without controversy. Bhermi et al.[18] cultured bovine LECs in a well-like poly(methyl methacrylate) pit. They reported that LECs migrated along the side wall out of the well, concluding that the rectangular circumstance did not induce contact inhibition of the LECs. The LECs in their study, however, might have been transformed after 6 or 7 cell culture passages. Furthermore, because the formed well was shallow, the possibility that the cells migrated out of the well when the cell layers reached the level of the well cannot be excluded, although in their illustration the single layer of LECs climbed out of the side wall of the well. It is generally accepted that with the exception of cancer cells, normal cells have, to date, not been observed to ascend the vertical well wall as contact inhibition of the cells is induced when they become confluent in the floor of the well.

Nagamoto and Fujiwara[19] grew cultured LECs on the surface of a bent collagen membrane. They concluded that contact inhibition was not induced by the rectangular configuration because the cells extended beyond the point of the bent line of the membrane. In their study, however, neither the bending technique of the collagen nor a description of whether the line was sharp or discontinuous could be established. Knowing these factors is important because if the bent line is not discontinuous LECs can migrate consistently over the allegedly discontinuous bent line.

In our study, LECs that migrated beyond the posterior capsule bend contained many Ki-67 positive nuclei, indicating that LECs were proliferating. This is in contrast to findings that LECs before the bend were Ki-67 negative. Capsular bending is experimentally and clinically complete within 2 to 3 weeks.[5,20,21] In vitro and clinically in vivo, LECs are known to migrate onto the posterior capsule within 1 week.[5,22,23] All these findings strongly suggest that the LECs found beyond the capsular bend on the posterior capsule migrated before the capsular bend was formed because once it forms, contact inhibition will be induced and LECs cannot migrate and accumulate before the bend. Therefore, the LECs observed beyond the bend are considered have migrated before the formation of the capsular bend.

The above findings and considerations strongly suggest that PCO in the eyes that receive an IOL with sharp edges is caused by LECs that migrate before the capsular bend is formed and from the site where the capsular bend fails to form. Therefore, PCO depends on the extent to which LECs migrate onto the posterior capsule before capsular bend formation and on the mitotic capacity of the cells, which is a function of the patient's age. Clinically, localized and limited PCO in the periphery of IOL optics can often be observed during the second or third postoperative years in older cataract patients. This PCO may be caused by LECs that migrated before the capsular bend was formed. Because their mitotic function was limited as a result of the age of the patient, they could not reach the visual axis and remained as a local accumulation of cells peripherally.

Therefore, it is the early postoperative period, the first or second postoperative week, that is the crucial period for the prevention of PCO. Bhermi et al.[18] and Nagamoto and Fujiwara[19] deny the possibility of "contact inhibition" and emphasize the importance of "compression." However, it not likely that an IOL causes compression to the posterior capsule within this time period as the human crystalline lens is more than 10 times (mathematically calculated) greater than the IOL in volume. Compression might happen in the presence of strong capsular fibrosis in the few months after surgery.

It was further observed that lens fiber cells (Soemmerring's ring) accumulated within the equatorial capsular bag and their growth appeared to have been stopped or hindered by the Ki-67 negative LECs that accumulated at the sharp optic edge. It is possible that because the LECs were contact inhibited, the subsequent LECs derived from the germinative zone were polarized and were transformed into lens fiber cells de novo, forming the so-called Soemmerring's ring cataract.

Another important question is whether Ki-67 negative LECs at the capsular bend will be reactivated in the future. In other words, will they again enter the cell cycle, undergoing mitosis, when the increasing Soemmerring's cataract abolishes the capsular bend? Clinical slitlamp observations indicate that LECs at the posterior optic edge do not migrate beyond the capsular bend despite elimination of the capsular bend by the bulky Soemmerring's ring, suggesting that Ki-67 negative LECs may stay permanently in the G_0 phase. However, years later, we have encountered the loss of the capsular bend as a consequence of Soemmerring's ring and PCO. Whether this PCO is the result of the reactivation of the LECs found in the G_0 phase or, as described, of the proliferation of the LECs that migrated before the capsular bend formed should be clarified in a future study.

It may appear inconsistent with findings that PCO-free capsules have been observed clinically on

occasion, even in eyes with a round-edged IOL. As shown in our previous studies,[24,25] sharp, blunt, or round edges are a relative notion and do not produce an all-or-nothing effect. Rather, they create a transition zone that may not necessarily be continuous. This may be an explanation for this clinical observation.

In conclusion, we found that the LECs at the capsular bend created by the sharp optic edge of an IOL were Ki-67 negative; that is, they were in the G_0 phase of the cell cycle. Thus, the posterior capsular bend formed induced contact inhibition of LECs in eyes that received an IOL with sharp edges, substantiating our theory of the effect of a sharp optic edge on PCO formation. Because LECs will not migrate once a capsular bend forms, PCO in eyes with a sharp-edged IOL is caused by the proliferation of the small amount of LECs that migrated onto the posterior capsule before the posterior capsular bend formed or they migrated through the site where the bending failed to form. Whether the LECs will be reactivated into the cell cycle from the G_0 phase when the capsular bend is abolished needs clarification in future studies. This concept of PCO pathogenesis contributes to the development of new strategies for the prevention of PCO.

REFERENCES

1. Nishi O, Nishi K, Mano C, et al. The inhibition of lens epithelial cell migration by a discontinuous capsular bend created by a band-shaped circular loop or a capsule-bending ring. Ophthalmic Surg Lasers 1998; 29:119–125
2. Nishi O, Nishi K, Sakanishi K. Inhibition of migrating lens epithelial cells at the capsular bend created by the rectangular optic edge of a posterior chamber intraocular lens. Ophthalmic Surg Lasers 1998; 29:587–594
3. Nishi O, Nishi K. Preventing posterior capsule opacification by creating a discontinuous sharp bend in the capsule. J Cataract Refract Surg 1999; 25:521–526
4. Nishi O, Nishi K, Wickström K. Preventing lens epithelial cell migration using intraocular lenses with sharp rectangular optic edges. J Cataract Refract Surg 2000; 26:1543–1549
5. Nishi O, Nishi K, Akura J. Speed of capsular bend formation at the optic edge of acrylic, silicone, and poly(methyl methacrylate) lenses. J Cataract Refract Surg 2002; 28:431–437
6. Abela-Formanek C, Amon M, Schild G, et al. Uveal and capsular biocompatibility of hydrophilic acrylic, hydrophobic acrylic, and silicone intraocular lenses. J Cataract Refract Surg 2002; 28: 50–61
7. Auffarth GU, Golescu A, Becker KA, Völcker HE. Quantification of posterior capsule opacification with round and sharp edge intraocular lenses. Ophthalmology 2003; 110:772–780
8. Buehl W, Findl O, Menapace R, et al. Effect of an acrylic intraocular lens with a sharp posterior optic edge on posterior capsule opacification. J Cataract Refract Surg 2002; 28: 1105–1111
9. Kruger AJ, Schauersberger J, Abela C, et al. Two year results: sharp versus rounded optic edges on silicone lenses. J Cataract Refract Surg 2000; 26:566–570
10. Schauersberger J, Amon M, Kruger A, et al. Comparison of the biocompatibility of 2 foldable intraocular lenses with sharp optic edges. J Cataract Refract Surg 2001; 27:1579–1585
11. Buehl W, Menapace R, Sacu S, et al. Effect of a silicone intraocular lens with a sharp posterior optic edge on posterior capsule opacification. J Cataract Refract Surg 2004; 30:1661–1667
12. Findl O, Menapace R, Sacu S, et al. Effect of optic material on posterior capsule opacification in intraocular lenses with sharp-edge optics; randomized clinical trial. Ophthalmology 2005; 112:67–72
13. Buehl W, Findl O, Menapace R, et al. Long-term effect of optic edge design in an acrylic intraocular lens on posterior capsule opacification. J Cataract Refract Surg 2005; 31:954–961
14. Rose DS, Maddox PH, Brown DC. Which proliferation markers for routine immunohistology? A comparison of five antibodies. J Clin Pathol 1994; 47:1010–1014
15. Gerdes J. Ki-67 and other proliferation markers useful for immunohistological diagnostic and prognostic evaluation in human malignancies. Semin Cancer Biol 1990; 1:199–206
16. Nishi O. Effect of a discontinuous capsule bend [letter]. J Cataract Refract Surg 2003; 29:1051–1052
17. Nishi O. Does a sharp edge compress the posterior capsule in vivo? [letter]. J Cataract Refract Surg 2005; 31:250–252
18. Bhermi GS, Spalton DJ, El-Osta AAR, Marshall J. Failure of a discontinuous bend to prevent lens epithelial cell migration in vitro. J Cataract Refract Surg 2002; 28:1256–1261
19. Nagamoto T, Fujiwara T. Inhibition of lens epithelial cell migration at the intraocular lens optic edge; role of capsule bending and contact pressure. J Cataract Refract Surg 2003; 29: 1605–1612
20. Hayashi H, Hayashi K, Nakao F, Hayashi F. Elapsed time for capsular apposition to intraocular lens after cataract surgery. Ophthalmology 2002; 109:1427–1431
21. Sacu S, Findl O, Linnola RJ. Optical coherence tomography assessment of capsule closure after cataract surgery. J Cataract Refract Surg 2005; 31:330–336
22. Nishi O. Incidence of posterior capsule opacification in eyes with and without posterior chamber intraocular lenses. J Cataract Refract Surg 1986; 12:519–522
23. Wormstone IM, Liu CSC, Rakic J-M, et al. Human lens epithelial cell proliferation in a protein-free medium. Invest Ophthalmol Vis Sci 1997; 38:396–404
24. Nishi O, Nishi K, Akura J, Nagata T. Effect of round-edged acrylic intraocular lenses on preventing posterior capsule opacification. J Cataract Refract Surg 2001; 27:608–613
25. Nishi O, Nishi K. Preventive effect of a second-generation silicone intraocular lens on posterior capsule opacification. J Cataract Refract Surg 2002; 28:1236–1240

First author:
Okihiro Nishi, MD

Nishi Eye Hospital, Osaka, Japan