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Ablation of lens epithelial cells with a laser photolysis system: Histopathology, ultrastructure, and immunochemistry

Nick Mamalis, MD, Hans E. Grossniklaus, MD, George O. Waring III, MD, Liliana Werner, MD, PhD, Jacob Brubaker, MD, Don Davis, MD, Ladan Espandar, MD, Rudolf Walker, PhD, and Reinhardt Thyzel

John A. Moran Eye Center (Mamalis, Werner, Brubaker, Davis, Espandar), University of Utah, Salt Lake City, Utah, and Emory Eye Center (Grossniklaus, Waring), Emory University, and a private practice (Waring), Atlanta, Georgia, USA; ARC Laser GmbH (Walker, Thyzel), Ekental-Forth, Germany

Abstract

PURPOSE—To evaluate efficacy of a neodymium:YAG (Nd:YAG) laser photolysis system in removing lens epithelial cells (LECs) and characterize the effect of the laser on laminin and fibronectin involved in LEC adhesion and migration.

METHODS—Cadaver eyes were evaluated using the Miyake technique. The lenses were removed with phacoemulsification. The modified Nd:YAG laser was used to clean the LECs from the capsule. Only the fornix was cleaned in some eyes and the anterior subcapsular area in other eyes. Some areas were not treated and acted as controls. Standard irrigation/aspiration (I/A) removal of LECs was performed in additional eyes. The eyes were analyzed using light microscopy and immunohistochemical staining.

RESULTS—Histopathologic evaluation showed that the laser removed the LECs from the anterior lens capsule and from the fornix. Immunohistochemical staining showed fibronectin and laminin staining in the untreated areas that was absent in the treated areas. Standard I/A removal of the LECs showed absence of cells but persistent laminin and fibronectin. Electron microscopy showed epithelial cells in untreated areas with an absence of the LECs and debris in treated areas.

CONCLUSIONS—The laser photolysis system removed LECs from the anterior lens capsule and capsule fornix. Along with the cells, laminin, fibronectin, and cell debris remained in the untreated areas but were removed by the treatment. This treatment may be useful in preventing posterior capsule opacification.

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Cataract surgery with intraocular lens (IOL) implantation has evolved into a highly successful procedure; however, opacification of the capsular bag, including posterior capsule opacification (PCO), remains the most common complication of modern cataract surgery. The incidence of PCO ranges from 10% to 30% in the first several years after cataract surgery. In 1998, Schaumburg et al.¹ published a metaanalysis of the published literature on PCO; they found the postoperative incidence of PCO to be 11.8% at 1 year, 20.7% at 3 years, and 28.5% at 5 years.

Corresponding author: Nick Mamalis, MD, John A. Moran Eye Center, University of Utah, 65 Mario Capecchi Drive, Salt Lake City, Utah 84132, USA. nick.mamalis@hsc.utah.edu.

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Significant PCO can be successfully treated by a neodymium:YAG (Nd:YAG) laser capsulotomy; however, the cost is substantial and there is the possibility of associated complications, such as postoperative intraocular pressure elevation, cystoid macular edema, and an increased incidence of retinal complications, including retinal detachment.^{1,2} It has been estimated that the overall expense of treatment of PCO in the United States is exceeded only by the cost of cataract surgery itself.³ With the continued development of specialty IOLs to correct astigmatism and to treat presbyopia with multifocal or accommodating IOLs, the development of capsule opacification and fibrosis with contraction becomes an even more disruptive complication. Furthermore, patients with so-called premium IOLs may require higher degrees of capsule clarity and subsequent earlier Nd:YAG laser capsulotomies that make the development of technologies to prevent capsule opacification imperative.

The major cause of PCO is the proliferation of lens epithelial cells (LECs) that remain in the capsular bag after phacoemulsification for cataract removal.⁴ Residual LECs can proliferate and subsequently migrate along the inner surface of the anterior and posterior lens capsule, clouding the capsule and in some cases causing fibrosis and contraction with subsequent reduction in vision and disruption of the position and function of the IOL.

Several methods have been used in an attempt to prevent PCO, including changes in IOL material and design. One of the most successful IOL design modifications was the incorporation of a sharp posterior optic edge, which when in apposition to the posterior lens capsule may have a barrier effect that prevents proliferating LECs from extending across the posterior lens capsule, causing PCO.^{5,6} Other methods aim to completely eliminate the LECs from the lens capsular bag using drugs, such as antimetabolites, toxic monoclonal antibodies, and other agents (eg, tranilast and ethylenediaminetetraacetic acid).^{7,8} Another method of removing LECs includes treatment with enzymes followed by aspiration.^{9,10} Furthermore, a sealed capsule irrigation system that allows complete closure of the capsulotomy, sealing the lens capsular bag, followed by injection of agents such as hypotonic water can cause the rupture or death of LECs, allowing them to be aspirated.^{11,12}

Mechanical methods of removing the LECs during cataract surgery include rough tips or round cannulas that manually scrape the cells off the anterior lens capsule and the fornix. Aspiration handpieces, such as the standard irrigation/aspiration (I/A) phacoemulsification instrument, can be used to remove LECs. The challenge to mechanical techniques is removing all cells, especially the regenerative ones around the capsular fornix; these cells cannot be visualized during surgery because of the presence of the iris.

A modified Nd:YAG handpiece has been used to remove LECs. The Nd:YAG laser handpiece was developed initially by Dodick et al.^{13,14} to use a fiber-optic bundle to transmit the Nd:YAG pulse to strike a small plate of titanium, resulting in a shock wave that emanates from a 0.8 mm opening in the tip with the initial purpose of removing soft lenses during cataract surgery. Dodick first proposed using this Nd:YAG laser photolysis system to remove LECs from the capsular bag (personal communication, Jack Dodick, MD, October 20, 2009). As of this writing, this instrument is not in active use for cataract removal. A modification of the laser photolysis system was developed (ARC Laser GmbH) to ablate LECs from the lens capsule. This modified Nd:YAG laser photolysis handpiece is coupled with the laser photolysis system, which is not adaptable from a standard Nd:YAG laser in the clinic. Pollhammer et al.¹⁵ performed a preliminary study to evaluate the ability of the laser photolysis system to ablate LECs in a porcine eye model. Histologic evaluation of the anterior lens capsule after treatment in their study showed that the laser photolysis system could completely ablate LECs from the anterior lens capsule.

The present study was performed to evaluate the efficacy of the modified laser photolysis system in the removal of LECs and glycoproteins from the anterior lens capsule as well as the capsular fornix in a human cadaver eye model. Light microscopy (LM) and transmission electron microscopy (TEM) were used to evaluate the efficacy of the LEC removal compared with the efficacy of the control methods of standard I/A and no removal. Furthermore, the effect of the laser on glycoprotein molecules that are involved in the adhesion and migration of proliferating LECs, such as laminin and fibronectin, was evaluated by immunohistochemical analysis.

MATERIALS AND METHODS

Sixteen human cadaver eyes were obtained from eye banks in the United States within 72 hours of death. Twelve of the eyes were prepared for surgery using the modified Miyake technique.¹⁶ In each eye, the cornea and iris were removed to improve the view of the lens capsular bag. The removal was performed because postmortem corneas are difficult to clear completely and the iris does not dilate. The crystalline lens was removed using an open-sky central continuous curvilinear capsulorhexis, standard phacoemulsification of the nucleus, and I/A of the cortex. Retroillumination was used to highlight the presence of LECs in the fornix and anterior capsular areas of the remnant lens capsular bag.

A modified Nd:YAG laser photolysis handpiece was then used to disrupt and remove LECs from the remnant lens capsular bag under direct visualization of the operating microscope. This photolysis system uses an Nd:YAG laser with a wavelength of 1064 nm and a titanium target to generate a shockwave that emerges from a 0.8 mm opening near the tip of the probe that is held approximately 1.0 mm from the lens capsule. The laser beam does not emerge from the handpiece; only the shockwave does. The probe does not touch the lens capsule. A 1.2 mm outer diameter probe was used with variable laser pulse power from 5 to 9 mJ, pulse width from 4 to 6 nanoseconds, and pulse repetition rate from 3 to 10 pulses/second. Based on previous laboratory experiments (unpublished data), the shockwave extended to a radius approximately 3.0 mm from the tip.

Viewed anteriorly through the operating microscope and posteriorly in the Miyake setup on the television screen (Figure 1), the removal of the LECs was seen as represented by the disappearance of a gray layer. All procedures were recorded.

A series of patterns in 8 of the Miyake eyes was used to remove the LECs: (1) The laser was used to attempt to clean LECs from the fornix only for 360 degrees, leaving the cells in the anterior subcapsular area intact. (2) The laser was used to treat the entire anterior capsular area, leaving only the LECs in the fornix intact. (3) For 360 degrees, the laser was used to remove LECs from the fornix and the anterior subcapsular area. (4) Treatment of the anterior capsule and fornix were performed for 180 degrees, leaving the LECs undisturbed in the remaining 180 degrees to act as a control.

Four eyes were treated using a whole globe technique to more realistically assess the laser treatment through clear corneal incisions. The iris was once again removed to improve visualization of the capsular bag, but the cornea remained intact in these eyes.

Under the same direct visualization setup, 4 control Miyake eyes were evaluated using standard bimanual I/A to vacuum the LECs from the anterior capsule and lens fornix with the cornea and iris removed.

The tissue was preserved in 10% neutral buffered formalin after each procedure. The eyes that had treatment using the Miyake technique were then carefully removed from the glass slide and processed for standard histopathologic evaluation using a tissue processor (Tissue-

Tek VIP, Sakura Finetek USA, Inc.); this was followed by paraffin embedding and sectioning. The anterior segment of the eyes was then stained with hematoxylin–eosin (H&E) to evaluate the capsular bag and the presence of LECs. The whole globes underwent similar processing and staining.

Immunohistochemistry

For immunohistochemical staining, unstained slides were deparaffinized and heated at 95°F for 20 minutes, cooled to room temperature for 20 to 40 minutes, and washed with phosphate buffered saline (PBS). The slides were then blocked with hydrogen peroxide for 5 to 10 minutes and washed with PBS once again; the primary antibody was applied and the slides were incubated overnight. The slides were then washed with PBS 3 times, labeled with polymer-HRP (DAKO) for 2 hours, washed with PBS 3 times, and stained with amino ethylcarbazole for 5 to 30 minutes. The slides were then washed with double-distilled water, counterstained with hematoxylin for 1 minute, washed with double-distilled water, dried, and cover slipped. Primary antibodies evaluated included antilaminin, fibronectin, junctional adhesion molecules (JAM-1), ZO-1, smooth muscle actin, and tenascin.

Light and Transmission Electron Microscopy

Eyes for TEM were submitted in 2.5% glutaraldehyde in 0.1 M phosphate buffer and kept at 4°C. The specimens were then postfixed with 0.1 M cacodylate buffer and 1% osmium tetroxide. After standard dehydration, the specimens were embedded in epoxy resin. Semithin (1.0 µm) sections were cut, stained with uranyl acetate–lade citrate, and evaluated by a using a transmission electron microscope (JEOL II CX).

Histopathology specimens were evaluated using LM.

RESULTS

Light microscopy of histopathology specimens showed that in areas in which the laser treatment was performed, the Nd:YAG laser shockwave completely removed the LECs from the inner surface of the anterior lens capsule (Figure 2) and from the fornix (Figure 3). Nontreated areas had diffuse LECs under the anterior lens capsule and in the fornix.

Immunohistochemical analysis showed that in the normal untreated areas, the basement membrane area of lens epithelium and some LECs stained for laminin (Figure 4). The entire lens epithelium and the lens zonules stained for fibronectin (Figure 5). The intracellular lens junctions stained for ZO-1. Stains were negative for JAM-1 and tenascin. In areas in which the lens epithelium was treated with the laser shockwave but was not effectively removed, the LECs formed blebs, with cellular debris present at the base of the bleb. This area of cellular debris stained for laminin and fibronectin. In areas in which the lens epithelium was treated with the laser and completely removed, there was no staining for laminin or fibronectin (Figures 6 and 7). Areas of completely removed LECs and areas in which LECs were still present occurred because the shockwave's efficiency in cleaning the capsule can vary with different laser powers and with varying distance of the handpiece tip to the capsule surface. For experimental evaluation of the sufficient power level, the laser power was decreased to values of approximately 5 mJ. The distance of the handpiece tip to the capsule was controlled strongly; however, with low power levels, cleaning was ineffective when the handpiece tip was moved in the capsular bag. The most efficient cleaning was achieved with power levels exceeding 7 mJ.

Transmission electron microscopy of the untreated areas showed normal anatomy of the lens capsule, zonular attachments, and LECs (Figure 8). The areas in which the cells were treated but not removed had LECs with cellular debris and blebs (Figure 9). In areas in which the

LECs were treated by the laser shockwave and removed, cellular debris was largely absent (Figure 10).

In the experiments in which the bimanual I/A was performed without the laser for LEC removal, the basement membrane areas of the lens epithelium stained for laminin and fibronectin where the epithelium was intact (Figure 11). There was an area of disrupted cellular debris where the LECs were removed by the aspiration; this area stained positively for laminin (Figure 12) and fibronectin (Figure 13).

DISCUSSION

In our study of human eye-bank eyes, the modified Nd:YAG laser photolysis system removed LECs from the inner surface of the anterior lens capsule and the capsular fornix. This was confirmed by LM and TEM. In the control untreated areas, the normal anatomy of LECs and capsular bag were present on both LM and TEM. In areas in which the lens epithelium was treated but not effectively removed, small blebs formed within the LECs and cellular debris persisted at the base of the blebs. Similarly, in areas in which the LECs were treated with attempted aspiration using standard bimanual I/A handpieces, there was remnant cellular debris from the LECs where the epithelium was not completely removed. These findings show the importance of using laser treatment and I/A in combination to effectively treat and remove LECs. In contrast, areas of the lens capsule in which the LECs were treated by the laser photolysis system were completely clear, with no LECs or cellular debris on LM or on TEM. In areas treated by the Nd:YAG photolysis system, neither laminin nor fibronectin was present on the surface of the lens capsule. In a clinical setting, this indicates that the cells would have no “carpet” on which to slide and migrate and no adhesion molecules upon which to take up residence.

Several methods to help prevent PCO have been evaluated, among them changes in IOL design. Nishi was one of the first researchers to propose that a square-edged IOL would prevent PCO by inhibiting the LEC migration.^{6,17} Nishi further observed that a change in the configuration of the posterior capsule occurred against a square edge of an IOL, which he termed capsular bend. This configuration, a discontinuous sharp square bend with tight wrapping of the posterior capsule around the sharp posterior lens optic, effectively inhibits LEC migration onto the posterior lens capsule.¹⁸ However, this approach requires 360-degree apposition of the posterior surface of the IOL to the lens capsule, which has not been achieved with all sharp-edged IOL designs. It is important for modern IOLs with sharp edges to have a design that incorporates a sharp posterior edge for 360 degrees without interruption by the haptics. Today, modifications allow these IOLs to be manufactured from many types of materials.

Various pharmacologic agents injected into the capsular bag have been used to prevent LEC proliferation and subsequent PCO. These include mitomycin-C,¹⁹ 5-fluorouracil,^{20,21} and daunomycin.²² Recently, tranilast microspheres in a sustained-release formulation were used to inhibit and further reduce PCO in an animal model.²³ The concept of using a system that allows sealed irrigation of the lens capsular bag and isolates the capsular bag from the remainder of the anterior segment permits targeting of LECs using pharmacological agents while minimizing the risk for damage to other intraocular structures. Maloof et al.¹¹ developed a system that uses a device that attaches to the anterior capsule opening by suction and provides a closed system for the introduction of agents directly into the capsular bag to treat LECs. This concept was evaluated in an animal model in which a sealed capsular irrigation device was used, followed by injection of a very toxic agent (triton $\times 100$). In animals in which the capsular bag was sealed, histopathologic evaluation showed no evidence of collateral damage to the anterior segment; however, significant damage to

the cornea, iris, and peripheral retina was noted when the material was injected directly into the anterior capsule. These findings suggest that the sealed capsular irrigation device allows selective delivery of toxic agents directly into the capsular bag while preventing collateral damage to surrounding intraocular tissues.¹²

Less toxic materials injected into the bag for treating LECs include a short exposure of LECs to distilled deionized water, which induces extensive and rapid cellular lysis of LECs. This could be used in a sealed capsule irrigation device to prevent capsule opacification.²⁴ However, it has not been effective in clinical use. For example, in a prospective controlled clinical trial using a device for sealed capsule irrigation with distilled water with a 2-year follow-up, Rabsilber et al.²⁵ concluded that “it is not possible to reduce PCO development significantly.”

Opacification of the posterior capsule after cataract surgery is the result of posterior migration of LECs. Basement membrane and extracellular matrix (ECM) glycoproteins may promote LEC migration and adhesion, which can play a role in the development of PCO. Studies using rabbit LECs found that the migration and adhesion of LECs occur in response to lens capsule proteins type IV collagen and laminin as well as in response to fibronectin. In fact, in 1 study,²⁶ fibronectin promoted maximum LEC migration.²⁶ Integrin adhesion receptors on cells can bind fibronectin and gain traction, allowing the LECs to migrate. Laminin is a major constituent of the lens basement membrane. Fibronectin is usually not found in the adult lens capsule but can be introduced into the anterior segment of the eye secondary to the inflammatory response after cataract surgery. In addition, fibronectin is produced by LECs that have transformed into myofibroblasts during inflammation. Fibronectin is also thought to be a mediator for adhesion between an IOL and the capsule.²⁷

Fibronectin has a functional domain to bind it to collagen. Collagen type IV is a typical basement membrane component of the lens capsule, the thickest basement membrane in the body. Because the lens capsule is made of collagen,²⁸ fibronectin could be a mediator for adhesion. Studies show adhesion of fibronectin, laminin, and collagen type IV to the surface of IOLs. These ECM components have also been observed in the connective tissue that accumulates between the capsule and the IOL.²⁷ Furthermore, Linnola et al.^{29,30} found that these adhesion-type molecules are found in a sandwich-like structure between the lens capsule and the surface of IOLs. This so-called sandwich theory may be involved in the decreased PCO noted in eyes with a hydrophobic acrylic IOL in the capsular bag.

Clinical studies of the use of the modified Nd:YAG laser photolysis capsule cleaning device in cataract surgery found the laser was successful in removing LECs from the anterior portion of the capsular bag (unpublished data). Briefly, the inner surface of the nasal half of the capsule in 17 eyes was treated with the laser after standard phacoemulsification under direct visualization through the dilated pupil; the temporal half of the bag was not treated. When one half the capsular bag was treated, the anterior and posterior capsule remained clear in most eyes for 2 years; the untreated half of the capsular bag became opaque within a few months after surgery. In the clinical series, treatment with the laser photolysis system not only prevented the capsule from opacifying in the areas that had treatment, but also prevented LEC migration onto the posterior capsule, preventing PCO from spreading from the untreated areas of the capsule to the treated capsule.

Presumably, if fibronectin and laminin are present on or in the lens capsular bag, residual LECs that are not removed from the lens capsule can migrate across an area of previously clear capsule and form capsule opacification, as often occurs commonly after the anterior capsule is cleaned using I/A.

Evaluation of control and untreated areas in our human eye-bank eye study showed that the lens capsule basement membrane area of the intact LECs, as well as some epithelial cells themselves, showed positive staining for laminin. In addition, the entire lens epithelium and some zonular fibers stained positive for fibronectin. In areas in which the LECs were treated with Nd:YAG photolysis but were not aspirated or removed, the areas of remnant cellular debris stained positive for laminin and fibronectin. Furthermore, in areas in which LEC removal was attempted using bimanual I/A, there was remnant cellular debris that stained positively for fibronectin and laminin.

The results in our study clearly show that the laser photolysis system facilitates complete removal of LECs from the inner surface of the anterior lens capsule as well as the capsular fornix without leaving LEC debris. Immunohistochemical staining clearly showed the absence of laminin and fibronectin in treated areas, and this may inhibit the proliferation of residual LECs along the treated lens capsule and prevent the subsequent generation of PCO. Use of aspiration without the laser left LEC debris, which contains laminin and fibronectin and may be involved in reepithelialization of the capsule and the onset of PCO. The Nd:YAG laser photolysis system is a promising treatment for the removal of LECs as well as adhesion and migration of glycoproteins, and this may prevent opacification of the capsular bag.

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Figure 1.
Removal of LECs from a human cadaver eye using the Miyake technique.

Figure 2. Photomicrograph shows the absence of LECs from the anterior subcapsular area after treatment with the laser photolysis system and with persistent LECs in the untreated fornix (arrow) (H&E, original magnification $\times 40$).

Figure 3. Photomicrograph of the lens capsular bag shows successful removal of LECs from the fornix after treatment with the laser photolysis system and persistent cells in the untreated anterior subcapsular area (*arrow*) (H&E, original magnification $\times 40$).

Figure 4. Immunohistochemical staining of the lens capsular bag and LECs from an untreated area shows positive staining in the basilar area for laminin (laminin, original magnification $\times 100$).

Figure 5. Immunohistochemical staining of an untreated area of the capsular bag shows positive staining of LECs and the zonules for fibronectin (fibronectin, original magnification $\times 100$).

Figure 6. Immunohistochemical staining of the lens capsular bag in which the LECs were treated with the laser photolysis system and completely removed shows no staining for laminin (laminin, original magnification $\times 100$).

Figure 7. Immunohistochemical staining of the lens capsular bag in which the LECs were treated with the laser photolysis system and removed shows no staining for fibronectin (fibronectin $\times 100$).

Figure 8.
Electron microscopy of an untreated control area shows normal anatomy of the LECs, lens capsule, and zonular attachments (TEM, original magnification $\times 4750$).

Figure 9. Electron microscopy of an area in which the LECs were treated with the laser photolysis system but were not removed shows areas of cellular debris (TEM, original magnification $\times 180$).

Figure 10.

Electron microscopy of an area in which the LECs were treated by the laser photolysis system and completely removed shows a relatively clean lens capsule where cellular debris is largely absent.

Figure 11. Immunohistochemical staining of an area in which the LECs were treated by bimanual I/A shows areas in which the basement membrane of the remnant LECs stains positive for laminin (laminin, original magnification $\times 250$).

Figure 12. Immunohistochemical staining of an area in which the LECs were removed by bimanual aspiration shows positive staining for laminin in the basilar area (laminin $\times 250$).

Figure 13. Immunohistochemical staining of an area in which the LECs have been removed by bimanual aspiration shows areas of positive staining for fibronectin in remnant cellular debris (fibronectin, original magnification $\times 250$)