CO$_2$ Laser-assisted Sclerectomy Surgery Part I: Concept and Experimental Models

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Purpose: To evaluate the safety and performance of a second-generation device for CO$_2$ laser-assisted sclerectomy surgery system in experimental models.

Materials and Methods: Laser-assisted deep sclerectomy using a modified CO$_2$ laser system (OT-134—"IOPtiMate"; IOPtima Ltd, Israel) was performed in 3 experimental models: enucleated pig eyes, human cadaver eyes, and live rabbit eyes. A half-thickness scleral flap was created, and a CO$_2$ laser with a beam-manipulating system was used to achieve deep scleral ablation over the Schlemm canal zone. Aqueous percolation and scleral perforation rates were recorded. Intraocular pressure was monitored in live rabbits up to 21 days postoperatively. The shape and location of the scleral ablation zone, thermal damage, and the healing process were examined by histopathological analysis.

Results: Deep scleral ablation and aqueous percolation were repeatedly achieved in all the models. Micro-perforations occurred in 4/18 human eyes (22.2%), in 4/23 rabbit eyes (17.4%), and in none of the 38 porcine eyes. Mean intraocular pressure in the rabbits was significantly decreased (by 6.3 ± 3.6 mm Hg) on the first postoperative day ($P < 0.0001$) and gradually returned to normal. In all but one of the cadaver eyes, effective fluid percolation was achieved. Histology in each case disclosed deep scleral craters with a thin intact sclero-corneal tissue layer at the ablation area. Mild thermal damage, limited to the ablated scleral walls was detected and resolved after 10 days.

Conclusions: The results in these experimental studies indicated that CO$_2$ laser-assisted sclerectomy surgery using the OT-134 system is a safe and efficacious procedure for achieving effective fluid percolation.

Key Words: glaucoma, filtration, nonpenetrating, deep sclerectomy, laser surgery, CO$_2$ laser

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C O$_2$ laser-assisted sclerectomy surgery (CLASS) is a proposed filtering procedure for the management of medically uncontrolled glaucoma. It offers an alternative to the penetrating trabeculectomy procedure, and is clinically similar to the manual procedure of nonpenetrating deep sclerectomy (NPDS). The latter, a procedure in current clinical use, is known to be associated with a reduced rate of postoperative complications,[1–9] but is technically rather difficult to perform. The manual operation starts with the creation of a scleral flap, followed by dissection and removal of a deeper, secondary partial-thickness limbal-based flap. Schlemm canal and the trabecular meshwork are then manually unroofed, allowing aqueous humor to percolate through the exposed trabeculo-Descemet membrane. A frequent complication of manual NPDS, however, is inadvertent perforation into the anterior chamber, necessitating conversion to a penetrating filtration procedure. Another frequent problem with NPDS, also related to technical difficulties, is insufficient tissue dissection, preventing effective fluid percolation and intraocular pressure (IOP) reduction.[9–11] A relatively long learning curve, high surgical skills, and considerable experience are therefore required to consistently achieve good clinical results.

CO$_2$ laser irradiation, because of its unique characteristics, was suggested by Assia et al[10,11] as a means of simplifying NPDS. Advantages of introducing this laser technique as part of the surgical procedure are the CO$_2$ laser-associated photoablation of dry tissue and coagulation of bleeding vessels, as well as the effective absorption of laser energy by any water or aqueous solution present, even if only in a minimal amount. After the superficial flap is created, repeated laser applications cause progressive ablation of thin layers of scleral tissue until aqueous percolation is achieved. The percolating fluid absorbs the laser energy, preventing it from reaching the sclera. The ablation therefore ceases "automatically" and penetration through the remaining thinned scleral wall is avoided. CLASS procedure thus substantially simplifies the nonpenetrating operation, while retaining its safety characteristics.

Preliminary studies of CLASS in animals and in human cadaver eyes,[10] as well as pilot clinical studies of CLASS with the first model of the CO$_2$ laser system (OT-133),[11] have demonstrated its remarkable efficacy in achieving fluid percolation with significant IOP reduction and a low perforation rate. In these clinical trials, performed in a series of 23 eyes, the IOP dropped from a mean of 27.2 mm Hg preoperatively to 11.3 mm Hg at 1 week and 16.9 mm Hg at 6 months postoperatively. However, 6 patients required medications and 3 patients who failed to improve required conventional trabeculectomy. Overall, CLASS using the OT-133 was found to be a safe and relatively easy procedure, but there were also several drawbacks, such as excessive charring and tissue coagulation around the treated area. Clinically it was evident that failure in several cases had occurred because of early adhesions and synechiae formation.

A second-generation system was then developed and constructed (OT-134—"IOPtiMate"; by IOPtima Ltd, Ramat-Gan, Israel) using a higher power laser and an advanced beam manipulator and scanner. Increasing the laser power and decreasing the beam dwell time on the scleral tissue resulted in increased control of the ablation process while decreasing residual momentary heating and tissue coagulation. The objectives of the present study were to evaluate the safety and performance of the OT-134 system in performing CLASS in ex vivo and in vivo laboratory models.

MATERIALS AND METHODS

CO_2 Laser and Beam-manipulating System

Scleral ablation was performed with the CO_2 laser system (40C; by Lumenis, Israel). The laser beam was focused and applied on the treated eye by the use of an OT-134 beam-manipulating system attached to an ophthalmic microscope (Fig. 1). The OT-134 consists of a scanner, used to control the shape, size, and scanning parameters of the focused laser beam, a micromanipulator that attaches the OT-134 system to the ophthalmic microscope and accurately positions the laser-scanned pattern on the desired ablation area, and a focusing assembly. Operation of the scanner is regulated by a control unit and the operating parameters are presented on a control panel.

The ablated area is demarcated by a red HeNe laser beam with approximately 200 micron spot size, which clearly indicates the boundaries of the targeted ablation area. In addition, the HeNe beam alternately marks the 4 corners of the targeted ablated surface.

Experimental Models and Surgical Procedures

CLASS was performed on ex vivo (porcine eyes and human cadaver eyes) and in vivo (rabbit eyes) laboratory models. The surgical procedure was similar in all models.

Surgical Procedure

The perilimbal conjunctiva and tenon capsule were removed and an anterior chamber maintainer was inserted to maintain an IOP of nearly 23 mm Hg (bottle height of 30 cm above the globe) in ex vivo eyes. A half-thickness 4×4 mm scleral flap was formed with a crescent knife.

A red laser (HeNe) aiming beam was used to mark the scanning area boundaries, with four clear red dots at the corners (Figs. 2A, 3A). Scan dimensions (width and length) could be changed within the range of 1 to 4 mm. Initially a wide scan area (eg, 2.4×2.0 mm) was used to repeatedly remove layers of sclera until the percolation zone could be readily identified by the clear signs of percolation. The ablation area was then reduced to 1.0 to 1.2 mm in the anterior-posterior direction, and adjusted to target the Schlemm canal region in human cadaver eyes, or the plexus area in pig eyes and rabbit eyes (Figs. 2B, 3B). The CO_2 laser was repeatedly applied with time intervals of 2 to 3 seconds between applications to allow percolation to take place and be detected, until sufficient percolation was clearly evident (Fig. 3C). The scleral flap was then repositioned and sutured with 10-0 nylon sutures.

Ex Vivo Models

Enucleated porcine eyes: CLASS procedure, using the OT-134 beam manipulating system, was first evaluated in enucleated porcine eyes. Assessments included aqueous percolation sufficiency and rate of macro-perforations
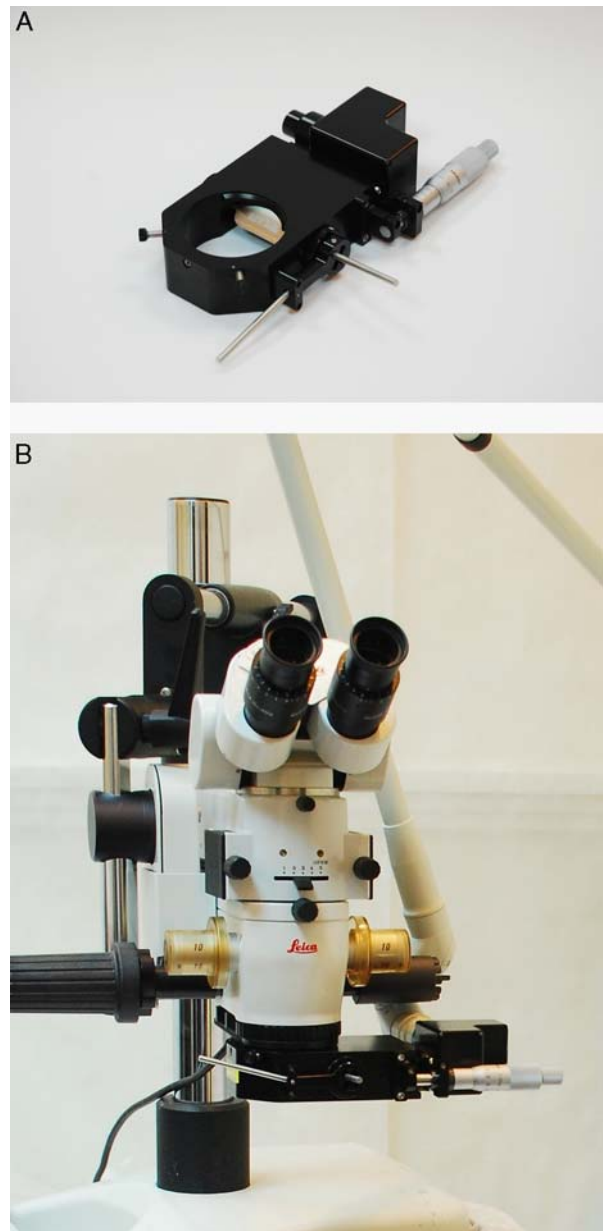
FIGURE 1. The OT-134 beam-manipulating system. A, The scanner, micromanipulator, focusing system, and adaptor are integrated as a single unit. B, The device is attached to an ophthalmic microscope. The articulated arm of the CO_2 laser is connected to the beam-manipulating system.

during scleral ablation. Clinical perforations were subjectively evaluated. Whereas a macro-perforation is an undesirable penetrating hole large enough to cause anterior chamber shallowing or iris prolapse, a micro-perforation is a full-thickness pinpoint hole that does not cause iris prolapse or shallowing of the anterior chamber, and is therefore not considered to be harmful. One to three procedures were performed on each eye. Laser power ranged from 20 to 28 W and the scanner was operated with a dwell time of 300 to 350 μs.

Aqueous humor drainage systems differ in animal and human eyes. Porcine and rabbit eyes, unlike human eyes,
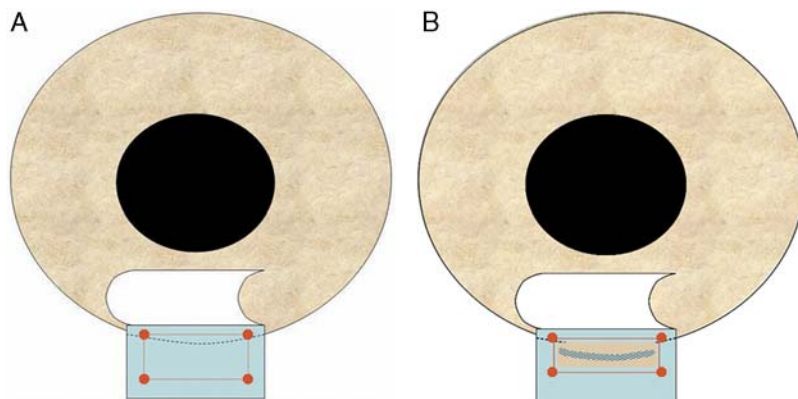
FIGURE 2. A diagram of the main anatomic landmarks in CLASS procedure. A, The scanned area corners are defined by 4 red HeNe laser dots. The anterior dots are aligned with the limbus under a scleral flap. B, The treated area (orange) may be narrowed and positioned over the Schlemm canal path (gray). CLASS indicates CO_2 laser-assisted sclerectomy surgery.

lack the Schlemm canal, and aqueous humor is drained through multiple tiny channels (plexus apparatus). However, the trabecular meshwork is a feature of both animal and human eyes, and percolation in both the models can be achieved by thinning of the scleral wall.

Human cadaver eyes: Two procedures were performed at different locations on each eye. A laser power of 24 W and scanner parameters of 300 μs dwell time were used in all cases.

The eyes were embedded in formaldehyde 4% and examined by staining with hematoxylin and eosin. Histological examination included evaluation of the scleral crater formed at the ablated sclera, assessment of the dimensions and integrity of the remaining trabeculo-Descemet membrane, and grading of the mechanical and thermal damage to treated and adjacent tissues.

In Vivo Model: Rabbit Eyes

The in vivo study was approved by the Committee for Ethical Conduct in the Care and Use of Laboratory Animals of the Hebrew University, Jerusalem. Twelve New Zealand White healthy male rabbits were divided into 4 subgroups according to the duration of follow-up (Table 1). Each rabbit underwent the CLASS procedure twice, once in each eye. A laser power input of 20 to 26 W (24 W in

20 procedures), and a dwell time of 300 μs were used for all the procedures.

Rabbits were anesthetized using intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg). Benoxinate 0.4% eye drops and 2% lidocaine gel were used for local anesthesia. Following fornix-based superior peritomy, CLASS was performed. Anterior chamber maintainers were not used in the in vivo study. The rabbits were treated postoperatively with Chloramphenicol 5% (Synthomycin, Rekah, Israel) and Dexametason 0.1% (Maxitrol, Alcon Laboratories, TX) ointments 3 times a day for up to 14 days, and were closely followed-up for up to 21 days.

Baseline IOP was measured on day 5, 3, and 1 before surgery using a pneumatonometer (Mentor, Model 30 Classic) under local anesthesia. An average of 3 measurements was recorded. IOP and clinical parameters were recorded during follow-up, and the rabbits were sacrificed at the end of their scheduled follow-up period. Student *t*-test was used to compare mean postoperative and baseline IOP for each time point. Repeated measures analysis of variance was used to examine the change in IOP over time. All eyes were examined histologically, with particular attention to the position and morphology of the ablated sclera, morphology of the adjacent tissues, inflammatory reaction, thermal damage at the ablated site, and the healing process.
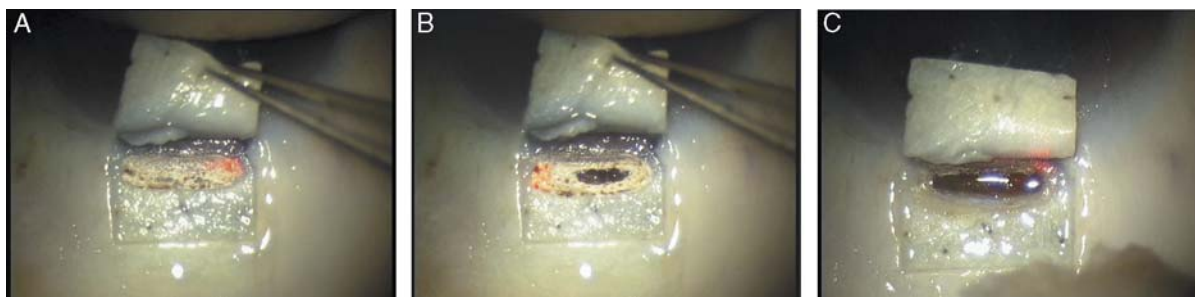
FIGURE 3. Photograph of CLASS procedure on a human cadaver eye. A, A red HeNe laser beam indicates the area selected for laser application, located on the gray zone between the clear cornea and the white sclera. In a captured picture, only 1 of the 4 dots can be seen. B, Schlemm canal location becomes visible as sclerectomy deepens, and initial aqueous percolation is evident. C, Once sufficient percolation is achieved, energy of additional laser applications is absorbed by the percolating fluid, preventing perforation and iris prolapse through the underlying thin residual tissue. CLASS indicates CO_2 laser-assisted sclerectomy surgery.

TABLE 1. Subgroups of In Vivo Rabbit Study

Group No.	No. Rabbits	No. Eyes	Follow-up Period
1	4	8	Immediately sacrificed
2	3	6	10 d
3	3	6	15 d
4	2	4	21 d

RESULTS

Porcine Eyes

A total of 38 CLASS procedures were performed in 25 enucleated porcine eyes. Satisfactory percolation was achieved in all cases, with no evidence of macro-perforations or micro-perforations.

Histological evaluation of 6 eyes (7 procedures) showed that the laser ablation had created a funnel-shaped scleral crater that was narrow at the desired percolation zone and wider at the crater's external opening (Fig. 4). The crater was located at the desired position over the plexus apparatus and the eye angle in all cases except for one, in which it was located slightly posterior to the plexus. In all the eyes, a thin layer of intact sclero-trabecular tissue was maintained at the percolation area.

In all the cases, the lateral walls of the crater showed a thin layer of thermal damage, 200 to 250 μm thick. Thermal damage was minimal or absent at the bottom of the crater, that is, over the exposed plexus where percolation takes place. None of the examined eyes showed any clinical or histological evidence of thermal damage to adjacent tissues such as the iris, ciliary body, or cornea.

Human Cadaver Eyes

Eighteen procedures were performed in 9 human cadaver eyes. Percolation was repeatedly achieved except in 1 case, in which laser ablation was too posterior. Schlemm canal could readily be detected in all the cases (Fig. 3).

Clinical micro-perforations occurred in 4 procedures (22%). In 2 of these cases, the ocular penetration occurred in late stages of the ablation as a result of mechanical injury with a wet Wekcell sponge that is used for cleaning and moistening the ablated tissue, and was not caused by the laser beam. The average number of laser applications was 32 (range, 12-53).

Histological examination in all cases disclosed a funnel-shaped scleral crater measuring 300 to 700 μm in width at its deepest part (Fig. 5). The position of the crater in relation to the draining system was too anterior in 2 cases and too posterior in 2 cases. In 7 procedures the residual tissue was seen discontinued, with iris prolapse in 1 case, although micro-perforation during the procedures was clinically evident only in 2 cases. Thermal damage was seen mainly on the lateral walls of the crater, and in 3 cases there was a mild damage to the Descemet membrane.

Rabbit Eyes

CLASS procedure was carried out in 23 eyes of 12 rabbits. One eye sustained inadvertent ocular penetration by the crescent knife during flap creation and the laser was not applied. Repeated percolation was satisfactorily achieved in 22 of the 23 treated eyes. In 1 eye, the laser application was too posterior to the limbus and did not achieve percolation. There were no device-related macro-perforations. Micro-perforation of the scleral wall occurred in 4 of the 23 eyes (17.4%).

All of the treated eyes showed mild conjunctival redness adjacent to the surgical incision, and a filtration bleb was evident in 3 eyes. In 1 eye, the anterior chamber appeared shallow on the day after surgery, but resolved spontaneously on the following day. The mean baseline IOP for all 24 eyes was 16.6 ± 2.8 mm Hg (mean $\pm$ SD). IOP dropped significantly 1 day after the surgery by 6.3 ± 3.9 mm Hg (n = 16, $P < 0.0001$), and steadily increased from day 2 to day 9 postoperatively, with IOP on the latter being similar to baseline. The incremental rise up to the ninth day was reflected in a significant linear trend ($P = 0.002$) modeled with repeated analysis of variance. There were no cases of persistent hypotony or ocular hypertension.
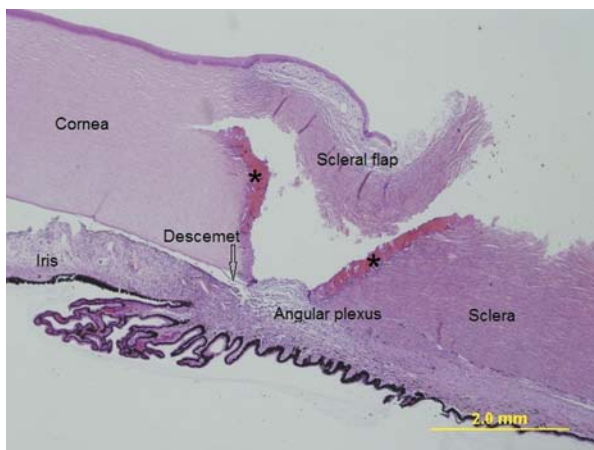
FIGURE 4. A, H&E staining of a porcine eye after CLASS. A scleral crater was produced over the percolating zone. Thermal damage is seen on the crater's lateral walls (asterisks; thickness: 250 μm). The tissue at the bottom of the crater is free of thermal damage. CLASS indicates CO_2 laser-assisted sclerectomy surgery; H&E, hematoxylin and eosin.
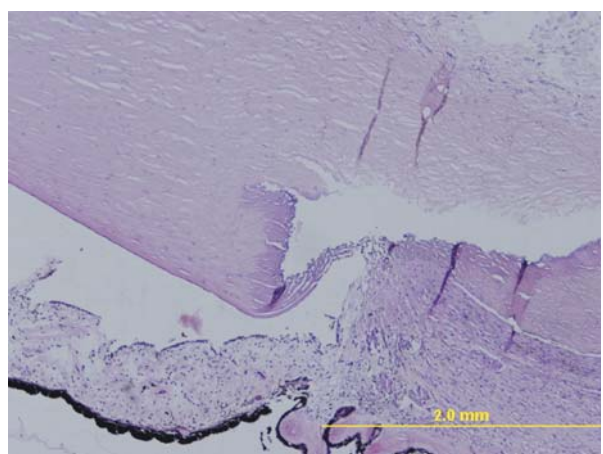
FIGURE 5. Histological section of a human cadaver eye after CLASS. A half depth scleral flap covers a residual thin intact layer overlying the trabecular meshwork and peripheral Descemet membrane. CLASS indicates CO_2 laser-assisted sclerectomy surgery.

In each of the rabbits sacrificed immediately after the surgery (7 eyes), histological examination disclosed a deep crater formed in the scleral wall down to the trabeculo-Descemet membrane. Blood, plasma, fibrin, and neutrophils filled the crater in some cases. Thermal damage to tissues at the crater walls and mild damage at the crater floor were seen in 4 cases. In the rabbits sacrificed 10, 15, and 21 days postoperatively, the crater was filled with loose connective tissue (Fig. 6). No inflammatory cells were seen in the anterior chamber or vitreous cavity.

DISCUSSION

Manual NPDS is currently used in clinical practice as a filtering procedure for the management of open-angle glaucoma. IOP reduction is achieved by facilitating outflow of aqueous humor through a thin intact trabeculo-Descemet membrane. Although such nonpenetrating procedures have high safety profiles, the surgical technique demands a high level of proficiency, and the efficacy relative to that of trabeculectomy is controversial.[3,5–9,12–14]

With the aim of simplifying the above deep sclerectomy procedure, various lasers have been suggested, including excimer and erbium:YAG lasers.[15–20] Certain inherent characteristics of the CO_2 laser make it suitable for use in filtration surgery. In particular, it effectively ablates the dry scleral tissue and is absorbed in water or aqueous solution within a very short penetration depth. Use of the CO_2 laser for the gradual removal of layers of scleral tissue leaves a thin intact scleral layer through which aqueous percolation occurs. At this stage laser energy is absorbed by the percolating fluid, protecting the remaining tissue from further ablation and undesired perforation.

The feasibility and safety of the CLASS using an early prototype, the OT-133 beam-manipulating system, were studied in experimental models and in clinical studies.[11] The OT-133 was superseded by the OT-134, which was designed to improve and refine control of the ablation process and further decrease residual laser-induced thermal damage to the tissue. The main difference between the OT-133 prototype and the revised OT-134 is in the laser power which is applied to the tissue (up to 28 W in the latter, compared with 18 W in the first prototype), which permits laser application within a shorter dwell time. The OT-134 also allows more working distance under the microscope (155 mm) and an improved resolution (0.2 mm compared with 1.0 mm) in controlling the scanning area pattern dimension.

In the present study, the OT-134 CO_2 laser system was evaluated in laboratory models. Adequate percolation at the drainage area was repeatedly achieved and histological sections demonstrated precise scleral ablation in all procedures. When the anterior edge of the laser-scan pattern was placed exactly on the exposed limbus, the Schlemm canal and trabecular area were readily and repeatedly identified at the borderline between the gray scleral tissue and the white sclera, facilitating exposure of the desired drainage area. The treated area was then reduced in the anterior-posterior direction of deeper ablations, resulting in a funnel-shaped crater in histological sections. Laser power of 24 W and dwell time of 300 µs were the most prevalent. No device-related technical complications were recorded. No learning curve was required, testifying to the simplicity of operating the laser system. There was no evidence of damage to adjacent tissues (sclera, cornea, iris base, and ciliary body). Although histological examination revealed
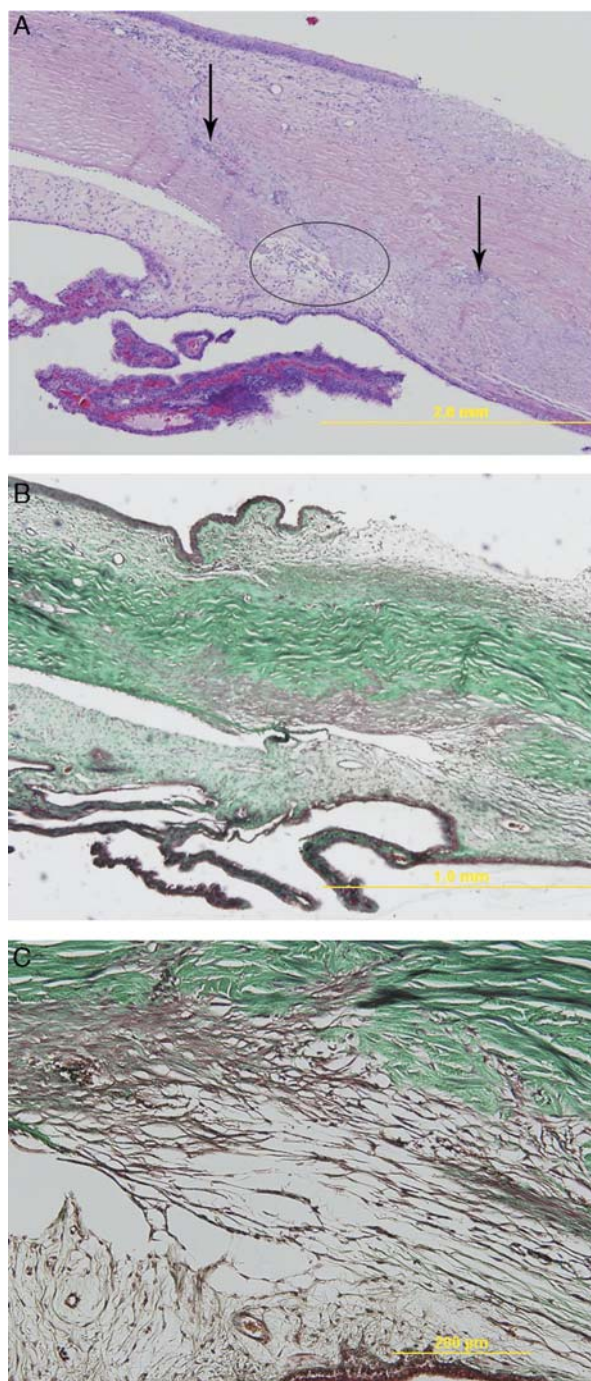
FIGURE 6. A rabbit eye 10 days after CLASS. A, H&E staining shows a fine basophilic line, the remnant of tissue that was thermally damaged by the laser application (arrows) and the percolation zone is encircled. B, Masson trichrome staining shows thin scar tissue at the scleral interface with no thermal damage. C, A high magnification of the treatment area shows loose tissue. CLASS indicates CO_2 laser-assisted sclerectomy surgery; H&E, hematoxylin and eosin.

scleral discontinuity with tissue prolapse in 3 rabbit eyes and in 1 human cadaver eye, no macro-perforations were recorded during any of these procedures. It is likely that the

prolapse occurred during processing of the histopathological specimens.

Clinical micro-perforations occurred in 4 cadaver eyes and in 4 rabbit eyes. Histological examination revealed additional cases of focal discontinuity of Descemet membrane. Small holes that are not associated with loss of anterior chamber depth and/or iris prolapse probably may improve aqueous drainage and do not necessitate conversion to penetrating trabeculectomy.[9] Some surgeons indeed advocate purposeful micro-puncturing of the canal roof with a needle to improve fluid flow. We do not recommend this practice, but at the same time we do not regard micro-perforation as harmful.

Transient thermal damage was evident on the lateral walls of the scleral crater in rabbits that were sacrificed immediately after surgery. In the live rabbit eyes, this tissue was replaced by a fine basophilic line overlying loose connective tissue 10 days postoperatively. At the actual percolation zone, there was little or no detectable thermal damage. Thermal damage is an integral effect of CO_2 laser as opposed to manual dissection or excimer laser, and was believed to be a major limiting factor in the success rate in the previous laser system. The current OT-134 was developed to decrease this undesired effect, by using a shorter dwell time of the laser beam.

Throughout the entire follow-up period, there was a consistent absence of inflammatory reaction in the adjacent tissues, the anterior chamber, and the vitreous cavity.

The in vivo animal study was chosen to assess safety parameters for the CLASS procedure. Although IOP was significantly reduced in all operated eyes 1 day after the surgery, it gradually returned to normal values within 9 days. The aim of the procedure in glaucoma patients is a sustained IOP reduction, whereas in rabbits a short-term reduction of the IOP was demonstrated. The transitory effect of surgery in rabbits may be explained by the enhanced inflammatory reaction and the intense healing process known to occur in rabbit eyes and the lack of a comparable structure to the human Schlemm canal. Moreover, pressure reduction in healthy rabbits with a normal IOP is more difficult to achieve. The single case of shallow anterior chamber with IOP of 0 mm Hg on the day after surgery resolved spontaneously on the following day, when the IOP reached 12 mm Hg. Micro-perforations occurred during surgery in this case. There were no cases of persistent hypotony or ocular hypertension during follow-up.

In summary, the feasibility and safety of CLASS using the OT-134 system were successfully demonstrated in laboratory models by the achievement of percolation with no evidence of macro-perforation. The results of subsequent clinical trials are reported in Part II of this paper.

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