EXCIMER LASER SCLEROSTOMY: THE *IN VITRO* DEVELOPMENT OF A MODIFIED OPEN MASK DELIVERY SYSTEM

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SUMMARY

The argon fluoride (ArF) excimer laser at 193 nm ablates the ocular tissues with a new order of precision and virtually no adjacent damage. A glaucoma filtration operation has been designed in which small-bore sclerostomies are created using the ArF excimer laser delivered through an open mask. The mask plicates the conjunctiva at the limbus prior to ablation. Removing the mask at the end of the procedure allows the conjunctiva to relax back to its original position, separating the conjunctival and scleral wounds. Formal conjunctival dissection is thus avoided. Feasibility studies in cadaver pig eyes, using a fluence per pulse of 400 mJ/cm² and a pulse repetition rate of 20 Hz, indicate that sclerostomies of 300 μm diameter can be reliably formed if an en-face air jet is built into the mask to raise the pressure in the target area, preventing aqueous flooding.

Trabeculectomy fails to maintain the intraocular pressure below 21 mmHg in 10–20% of cases depending on the series,[1] and this failure rate increases with repetition. In trabeculectomy, the factors which determine the initial filtration rate (operative dimensions and the tension of scleral flap apposition) are only notionally controlled. Tissue trauma and the subsequent wound healing response are also variable. Thus failure of filtration in a proportion of cases is difficult to avoid.

Reliability might be improved in glaucoma filtration surgery if the stimulus to wound healing from tissue trauma were minimised and operative dimensions were accurately controlled. The (193 nm) argon fluoride (ArF) excimer laser has been shown in corneal surgery to perform exceptionally well on both counts, ablating with sub-micro metre precision and virtually no damage to the adjacent tissues.[23]

Two approaches to glaucoma surgery using the excimer laser at 193 nm have already been described experimen-

tally: partial external trabeculectomy, in which ablation of a 2 mm × 0.8 mm rectangular scleral channel proceeds until increased aqueous efflux arrests progress with removal of the high resistance, endothelial portion of the trabecular meshwork;[4–7] and a modified conventional trabeculectomy procedure in which the excimer laser is used to smooth the surfaces beneath a conventionally dissected scleral flap, then to form a sclerostomy and a peripheral iridotomy.[8] Both these approaches require formal preparatory conjunctival dissection, however, and this in itself is a major element of the tissue trauma inherent in conventional procedures.

Sclerostomies can be formed without conjunctival dissection by a variety of lasers delivered *ab interno*, either endoscopically or gonioscopically.[9–20] *Ab interno* delivery is not possible with the 193 nm excimer laser, however, since light at this wavelength is strongly absorbed by the cornea and cannot be delivered endoscopically.

An *ab externo* approach in which conjunctival dissection is avoided has been conceived for the formation of small bore (≤500 μm) sclerostomies using the ArF excimer laser. This utilises a modified open mask to plicate the conjunctiva at the limbus prior to through-and-through ablation (Fig. 1a). Removal of the mask at the end of the procedure allows the conjunctiva to relax back to its original position, separating the conjunctival wound from the sclerostomy, and allowing a bleb to form (Fig. 1b). The development of a viable modified open mask system for excimer laser sclerostomy formation, and the results of ablation in cadaver pig eyes, are described below.

MATERIALS AND METHODS

Cadaver pig eyes stored at 4 °C and used within 12 hours of enucleation were mounted on a vacuum holder. Prototype masks were attached and aligned with the limbus as described above. The eyes were perfused with a constant pressure head of normal saline through an intravitreal cannula and left to equilibrate at 30 mmHg for 5 minutes prior

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Fig. 1a

Fig. 1b
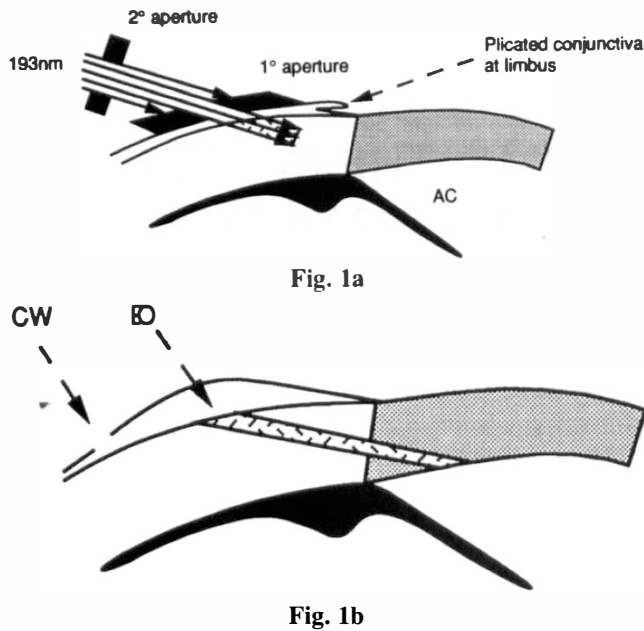
Fig. 1. *(a) A schematic illustration showing plication of the conjunctiva at the limbus by the main component of the open mask. This fixes the conjunctiva by gentle suction before being hitched forward and stabilised in the definitive position by attachment of a second vacuum component to the cornea (not illustrated). The angle at which the sclerostomy enters the anterior chamber (AC) is controlled by concentric alignment of primary and secondary apertures built into the main component. The primary aperture defines the cross-sectional outline of the sclerostomy. The position of the external ostium is controlled by alignment of the anterior edge of the main component of the mask with the limbus. (b) After ablation is complete and the mask is removed, the conjunctiva relaxes back, clearly separating the small conjunctival wound (CW) from the external ostium (EO) of the sclerostomy.*

to ablation. They were then positioned beneath an operating microscope, built into the laser delivery system, with a graticule (indicating the beam centre), the secondary aperture and the primary aperture aligned concentrically.

Using a Questek model 2820 ArF (193 nm) excimer laser with a delivery system configured to produce an even distribution of energy across a 1.5 mm rotating beam at the mask surface, ablation then proceeded at a fluence per pulse of 400 mJ/cm^2 and a pulse repetition rate of 20 Hz until penetration had occurred or for a maximum of 5000 pulses. Energy levels were checked prior to each experimental session as previously described.[21]

Intraocular pressure was monitored throughout using a Hewlett-Packard 78905A monitor with a pressure transducer connected to the anterior chamber via a closed fluid interface through a 27 gauge cannula entering opposite the ablation site.

Following ablation, the eyes were immediately perfused with 1 ml 2.5% glutaraldehyde via the intracameral cannula. The eyes were then immersed in 2.5% glutaraldehyde for a minimum of 24 hours before examination under a dissecting microscope. Dissected specimens were dehydrated with serially increased concentrations of alcohol and critical point drying prior to evaporative coating with gold and carbon. Further examination was then

performed with a Phillips 505 scanning electron microscope.

The prototype masks were progressively modified to examine the effect of the following air-flow conditions on sclerostomy formation: no air-flow modification, a venturi exhaust system (Fig. 2a), and an en-face jet (Fig. 2b). These modifications were designed to lower and raise the pressure within the ablation channel (i.e. the forming sclerostomy) beneath the mask's primary aperture. The pressure conditions generated were recorded using a water filled U-tube manometer with a rubber cuff on the end of one arm which, when compressed against the inferior aspect of the mask, formed an airtight seal allowing a steady-state measurement of the pressure beneath the primary aperture.

RESULTS

Three eyes were ablated with no air-flow modification. Constant seepage of aqueous fluid through the sclera into the ablation site was observed. Bright blue/violet fluorescence within the primary aperture was seen throughout ablation, and progress after 5000 pulses was limited in all eyes to a shallow conical indentation (estimated at less than 20% of scleral thickness) beneath the primary aperture. These specimens were examined under the dissecting microscope only.


Fig. 2a

Fig. 2b
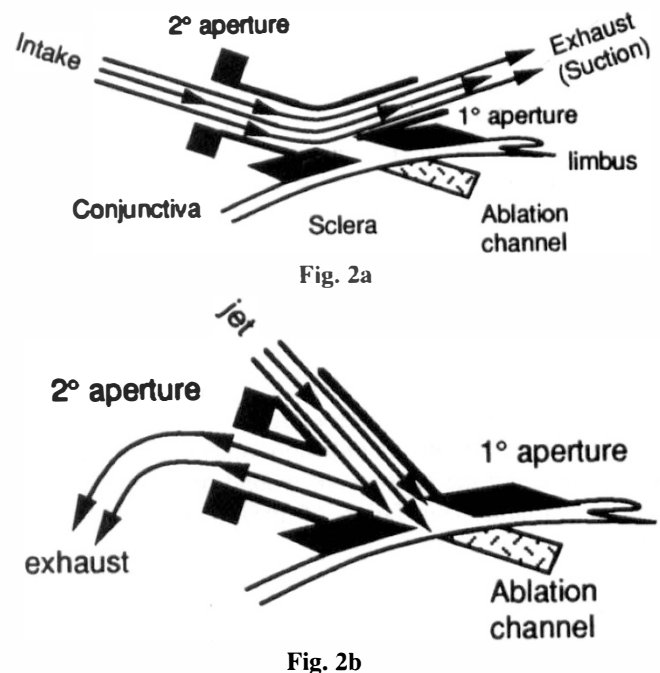
Fig. 2. *(a) The venturi exhaust system. Air is sucked through the primary aperture and out of the main component of the mask through an exhaust tube. A high-velocity stream of air is thus passed across the primary aperture (see arrows) creating a vacuum in the ablation channel below. The incident beam is directed through the secondary and primary apertures as illustrated in Fig. 1(a). (b) The en-face jet system. A jet of sterile air is passed almost directly down through the primary aperture (see arrows) raising the pressure within the ablation channel. Exhaust is through the secondary aperture across which an external current of air is directed to clear ablation products away from the path of the incident beam.*
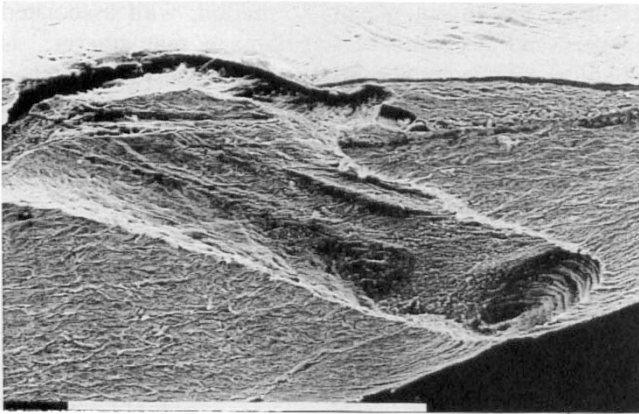
Fig. 3. *A 500 μm excimer laser sclerostomy formed using the venturi exhaust system, examined in cross-section. Scale bar represents 1 mm.*

Eight eyes were ablated using the venturi exhaust system creating a negative pressure of 95 cmH$_2$O beneath the mask. The primary aperture was 500 μm in diameter. Aqueous seepage into the ablation channel was not observed, and initial progress of ablation appeared to be good with a dark hole forming beneath the primary aperture. This became lucent in a characteristic manner as the sclerocorneal junction was passed and transillumination through clear cornea emerged like a new moon gradually becoming full. In three eyes, full-thickness perforation occurred after 4410, 2800 and 3610 pulses. This event was marked by the appearance of a fine fluid ejaculate streaming up the exhaust tube from the primary aperture. In eyes that did not perforate after 5000 pulses, a marked increase in blue/violet fluorescence was noted within the ablation channel at 1000–3000 pulses. Cross-sectional examination of the eye in which perforation occurred at 3610 pulses (Fig. 3) revealed a smooth-walled, cylindrical, 500 μm diameter sclerostomy with no apparent damage to the adjacent tissues. At the base, a terraced pattern of transected corneal lamellae was seen stepping down to an eccentrically placed site of full-thickness penetration. Examination of the eyes perforating at 4410 and 2800 pulses from the endothelial side indicated that this site
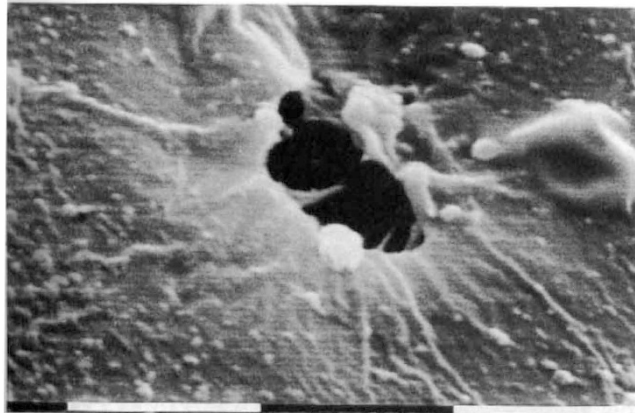
measured 10–15 μm in its largest dimension (Fig. 4a) and was surrounded by a zone of endothelial loss beneath the main body of the sclerostomy (Fig. 4b). Cross-sectional examination of the remaining eyes revealed sclerostomies similar to that illustrated (Fig. 3), but without a perforation site or a clear terraced lamellar pattern at the base, which included a variable thickness of residual stroma (<100 μm) superficial to Descemet's membrane. In one eye ablation appeared to have been arrested much earlier with the transection of an aqueous vein (Fig. 5).

Twenty eyes were ablated with a mask incorporating an en-face air jet. In the first five eyes, a high positive pressure (in excess of 150 mmHg) was used. The primary aperture was 500 μm in diameter. Ablation followed a similar pattern to that observed for the venturi system, but perforation occurred in all five eyes after 2050–2900 pulses (mean 2380) and was marked by the appearance of an air bubble in the anterior chamber. Examination again revealed smooth-walled sclerostomies but with an extensive zone of endothelial damage (0.5–2 mm) with splits in Descemet's membrane surrounding the internal ostia (Fig. 6).

The minimum en-face jet pressure at which fully penetrating sclerostomies could be obtained was then approximated by ablating five eyes with progressive 10 cmH$_2$O (7.4 mmHg) increments of pressure beneath the 500 μm mask commencing at 20 cmH$_2$O (14.8 mmHg). All eyes were exposed to 4000 pulses. Examination confirmed that fully penetrating fistulas were obtained where the pressure beneath the mask was 50 cmH$_2$O (37 mmHg) or greater. Ten eyes were then ablated with a positive pressure of 37 mmHg beneath the mask. The primary aperture was reduced to 300 μm. Full-thickness penetration occurred in all ten eyes, each exposed to 4000 pulses. The moment of perforation was marked by a sudden change in the retroilluminated base of the ablation channel, with or without the appearance of a small air bubble in the anterior chamber. With perforation, intraocular pressure quickly rose to equilibrate with the en-face jet pressure. Cross-sectional examination of three eyes showed 300 μm cylindrical sclerostomies with bases
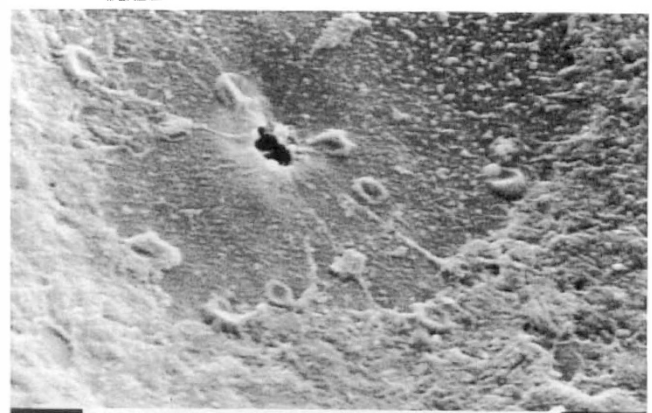
Fig. 4a

Fig. 4b

Fig. 4. *(a) The small perforation at the base of an excimer laser sclerostomy formed with the venturi exhaust system examined from the endothelial side. Scale bar represents 10 μm. (b) A lower-point view of the same specimen showing endothelial loss beneath the main portion of the sclerostomy. Scale bar represents 1 mm.*
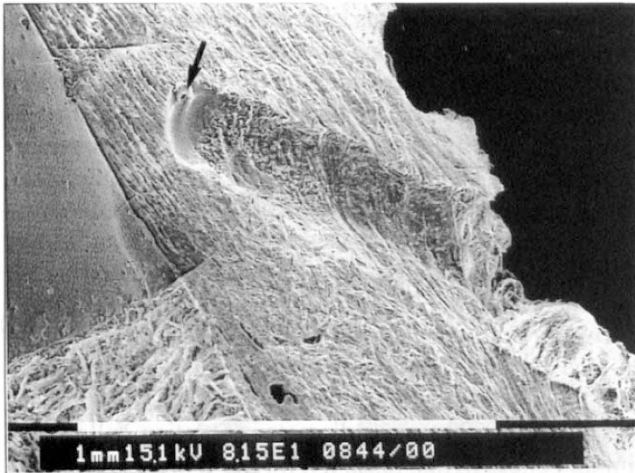
Fig. 5. *The base of an ablation channel. Progress of ablation appeared to have been arrested in this case by transection of an aqueous vein (see arrow). Scale bar represents 100 μm.*

truncated obliquely at a variable point by entry into the anterior chamber (Fig. 7a). There was no apparent adjacent tissue damage (Fig. 7b), although a fine fibrillar deposit was consistently seen along the lower edge of the cut section (Fig. 7c). The remaining seven eyes were examined from the endothelial side. Internal ostia were round or oval (Fig. 8) with a smallest diameter varying between 40 and 290 μm (mean 188; SD 92). Surrounding endothelial damage was restricted to a maximum radius of 100 μm. In many areas the endothelium was intact immediately adjacent to the ostium.

DISCUSSION

The pig eye was used as a test bed because it has similar dimensions to the human eye. In particular, the recess of the anterior chamber angle behind the surgical limbus in the sagittal plane is approximately equivalent.

Ablation parameters were chosen with a view to maximising the speed of sclerostomy completion whilst maintaining the minimal tissue damage zone. A fluence of 400 mJ/cm^2 was selected to produce a relatively high etch depth per pulse without compromising ablation effi-
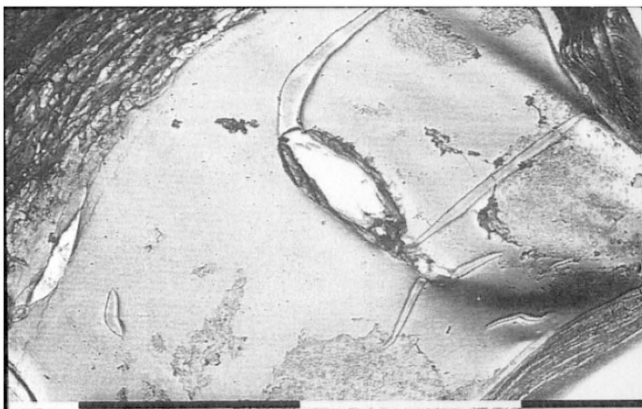
Fig. 6. *Extensive endothelial loss and splits in Descemet's membrane surrounding the internal ostium of a sclerostomy formed using a high-pressure (>200 cmH$_2$O) en-face jet. Scale bar represents 1 mm.*

ciency.[22] Significant plasma formation, with associated thermal and mechanical tissue damage, may emerge[22] as ablation efficiency diminishes at fluences greater than 400 mJ/cm^2. Although pulse repetition rates of up to 30 Hz[6] have been used in partial external trabeculectomy, thermal damage may begin to accrue[23] with pulse rates in excess of 20 Hz at 400 mJ/cm^2.

The immediate conclusion drawn from initial attempts at excimer laser sclerostomy formation was that, unless some provision is made for drying the ablation channel during the procedure, progress is quickly arrested by aqueous seeping in and absorbing the incident radiation.

The venturi exhaust system, in which a jet of dry air is passed over the top of the mask's primary aperture, was designed to encourage evaporation of pooled aqueous by lowering the ambient pressure within the ablation channel, and to conduct vapour away efficiently. This was only partially successful. Slow aqueous pooling during the early stages of ablation appeared to be compensated, but the vacuum later seemed to have the reverse of the desired effect, preventing full-thickness sclerostomy formation by sucking aqueous into the ablation channel.

The opposite approach of raising pressure within the ablation channel using an en-face jet solves this problem. Consistent perforation was obtained where pressure in the ablation channel exceeded intraocular pressure by approximately 10 mmHg. Intraocular pressure was maintained at 30 mmHg to simulate the likely conditions *in vivo* after attachment of a vacuum mask (placement of the prototype vacuum masks was found to raise the intraocular pressure by 6–11 mmHg). Use of the minimum en-face jet pressure avoids the extensive damage to Descemet's membrane seen where higher pressures are used and the sclerostomy bursts through into the anterior chamber. It also minimises the peak pressure which the eye is exposed to when the sclerostomy enters the anterior chamber and intraocular pressure equilibrates with the air jet. *In vivo*, there would be a theoretical risk of introducing infection through the air jet at this point; but the sterilising action of 193 nm irradiation[21,24] in conjunction with the use of filtered sterile air should minimise this.

For complete penetration in which the internal ostium matches the diameter of the main body of the sclerostomy, ablation for a number of pulses beyond the initial entry is required (since the base of the cylindrical ablation channel is not parallel to the endothelial surface). There might, however, be some advantage in leaving the sclerostomy guarded by a smaller internal ostium to reduce the incidence of complications associated with excessive aqueous outflow. Further studies are in progress to determine the number of additional pulses required for complete penetration and the limits of dimensional reproducibility for both guarded and unguarded sclerostomies produced with this delivery system.

Animal models of filtration surgery[25,26] indicate that fibroblast proliferation and cicatrisation in the subconjunctival space is the precipitating event in filtration failure rather than internal closure of the drainage fistula
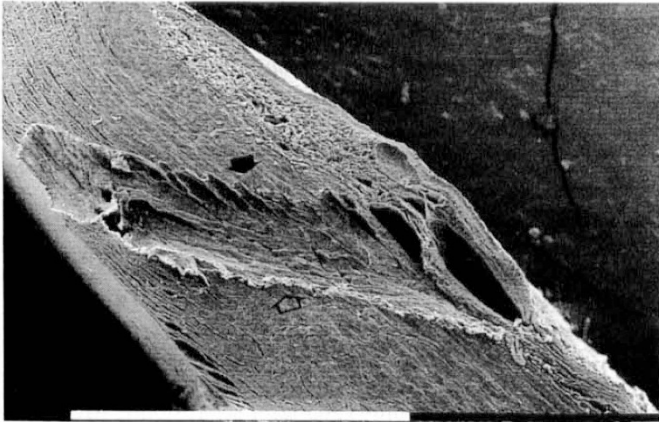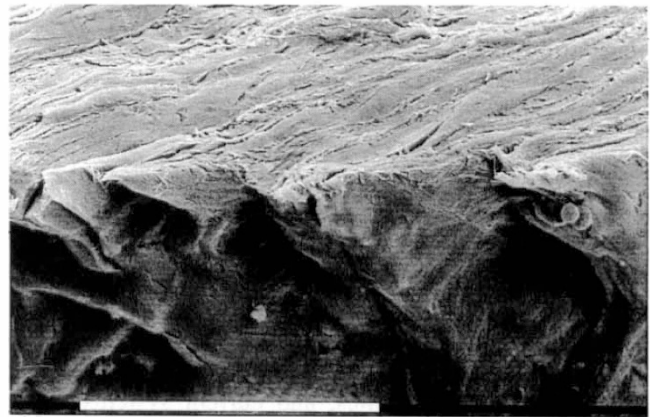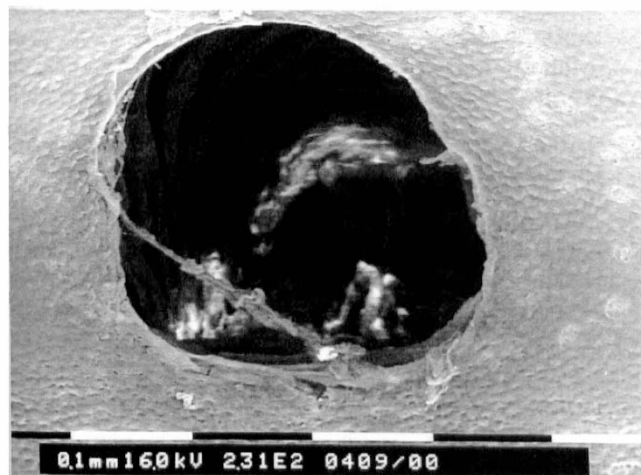
Fig. 7a

Fig. 7b

Fig. 7c

Fig. 7. *(a) A 300 μm excimer laser sclerostomy formed using a low-pressure (50 cmH₂O) en-face jet examined in cross-section. Scale bar represents 1 mm. (b) A high-power view of the middle portion of the upper edge of this specimen (see filled arrow in (a)) showing the lack of damage to tissues adjacent to the sclerostomy. Scale bar represents 100 μm. (c) A similar high-power view of the lower edge (see open arrow in (a)) showing a fine fibrillar deposit but again no manifest tissue damage zone. The fibrillar deposit may represent collagen bundles artefactually displaced by the downward sweep of the razor blade used to section the specimen.[5] Scale bar represents 1 mm.*

itself. This would suggest that sclerostomies considerably smaller than those created in conventional full-thickness filtration procedures might be equally effective in maintaining filtration.

Minimising tissue trauma and the stimulus to wound healing may assist in allowing smaller-bore fistulas to remain patent. Using the same contact endoscopic delivery system to create sclerostomies in rabbits with two

Fig. 8. *An excimer laser sclerostomy formed using a low-pressure (50 cmH₂O) en-face jet examined from the endothelial side, showing minimal disturbance to the adjacent endothelium. Scale bar represents 100 μm.*

different lasers, Hill *et al.*[27] noted that fistulas formed with the mid-infrared erbium laser were significantly more durable than those formed using a continuous wave Nd:YAG laser with its larger thermal tissue damage zone. Similarly, fistulas created in rabbits with a high-powered argon laser (thermal damage zone 250 μm)[9] all closed within 4 days, whereas those created with the XeCl excimer laser at 308 nm (thermal damage zone 50–100 μm)[18–20] remain patent for up to 4 months. The only clear evidence of damage to the surrounding tissues observed here using the excimer laser at 193 nm and a mask pressure of 37 mmHg was a variable small (<100 μm) zone of endothelial loss about the internal ostia. Open mask delivery *ab externo* allows free escape of the hot expanding gases created during ablation. This contrasts with current contact endoscopic delivery systems which trap these ablation products within the forming sclerostomy, causing significant secondary thermal and mechanical damage.[28]

Early experience of excimer laser sclerostomy *in vivo* indicates that conjunctival plication to separate the corneal and scleral wounds resulting from through-and-through ablation works well, and that functional filtration blebs with a self-sealing conjunctival wound can be formed. Although ablation with the ArF excimer laser is non-haemostatic (because of the absence of adjacent coagulative thermal tissue damage), haemostasis is maintained during the procedure by positive pressure tamponade from the en-face jet, and the duration of ablation

(approximately 4 minutes) exceeds the bleeding time for most of the small vessels transected. Simultaneous coagulation and haemostasis can be obtained in sclerostomy procedures using other lasers,[9,15] but only at the expense of a relatively large zone of thermal tissue damage.

Laser mechanisms and delivery systems used in sclerostomy procedures have been extensively reviewed in an earlier paper.[29] Excimer laser sclerostomy theoretically combines the least traumatic laser with the least traumatic delivery system. Accuracy is similarly optimised. Its use in therapeutic and refractive corneal procedures is likely to continue to propagate the availability of the ArF excimer laser in ophthalmic clinics, and current systems could easily be adapted to accommodate this new procedure, which may eventually offer a convenient, atraumatic and reliable alternative to conventional glaucoma filtration surgery.

Key words: Excimer, Filtration, Glaucoma, Laser, Sclerostomy, Surgery.

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