**SCIENTIFIC REPORT**

**Influence of non-penetrating glaucoma surgery on aqueous outflow facility in isolated porcine eyes**

T Shaarawy, R Wu, A Mermod, J Flammer, I O Haefliger

**Purpose:** To investigate, in vitro, the influence of non-penetrating glaucoma surgery (NPGS) and the influence of tightly suturing the superficial scleral flap on the aqueous outflow facility of isolated porcine eyes.

**Materials and methods:** The anterior chambers of 12 enucleated porcine cadaver eyes were cannulated and perfused. NPGS was performed by the same surgeon. The overall aqueous outflow facilities were assessed before and after the surgical interventions of NPGS, as well as after scleral flap closure.

**Results:** The mean (SD) aqueous outflow facility, which was 0.164 (0.014) µl/min/mm Hg before surgery, increased significantly after NPGS to 1.384 (0.217) µl/min/mm Hg, p<0.001. When the superficial flap was closed, the aqueous outflow facility significantly decreased (0.754 (0.107) µl/min/mm Hg, p<0.001) but remained significantly higher than preoperatively (p<0.01). After suturing the superficial flap, the overall resistance increased to 1.625 (0.210) µl/min/mm Hg. The difference in the resistance to outflow before and after flap closure was 0.848 (0.169) µl/min/mm Hg.

**Conclusion:** After NPGS suturing the scleral flap can modulate aqueous outflow resistance. The experimental set up described might provide an efficient model for the technical training of glaucoma surgeries.

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*Non-penetrating glaucoma surgery (NPGS) is a relatively modern type of surgery that includes different methods, the most popular of which are deep sclerectomy (DS) and viscoscanalostomy. The main idea behind NPGS is to target the portion of the aqueous outflow pathway responsible for the main resistance to outflow, and to create filtration through the naturally occurring trabeculo-Descemet’s membrane (TDM). The TDM thus offers a relatively important bulk of the overall resistance to outflow in the operated eye, and because it is a naturally occurring membrane the resistance offered is reproducible. One of the main differences between DS and viscoscanalostomy is that the proponents of the latter technique advocate tight closure of the superficial scleral flap.

Most of the available reports on NPGS comment on efficacy and safety, with very few studies published on potential mechanisms of function. The purpose of this study is to explore the effect of NPGS on outflow facility, as well as to investigate the effect of tight closure of the superficial scleral flap on the outflow facility after NPGS.

**MATERIALS AND METHODS**

In adherence with the ARVO statement for the use of animals in vision research, pig eyes were obtained from a local abattoir immediately after the animal’s death. The eye was fixed on a support device and the anterior chamber was cannulated with a 27 gauge catheter (fig 1). The catheter was placed between the anterior plane of iris and the inner surface of the cornea. The catheter was then connected to a microsyringe pump which allows stable and continuous perfusion to the anterior chamber in various flow rates. This perfusion arrangement was connected to an electronic pressure transducer by a stopcock. The whole system was filled with modified Krebs-Ringer solution. During the whole procedure of the outflow facility assessment and the surgery intervention, intraocular pressure (IOP) of the eye was monitored continuously by a pressure monitor (BP-1, WPI, Sarasota, FL, USA).

**Outflow facility assessment**

The anterior chamber was perfused at first with a flow rate of 5 µl/min. After a stable IOP level was reached, the flow rate was changed to 10 µl/min until another stable IOP level was reached. The value of the change in flow rate divided by the IOP change measures represents outflow facility (C). The outflow facility is thus calculated using the Goldman equation

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C = \Delta I/\Delta IOP \quad (\Delta I = I_2 - I_1)
\]

where \(I_1\) and \(I_2\) are successive inflow rates (µl/min), \(\Delta IOP = (P_2 - P_1)\), where \(P_1\) and \(P_2\) represent IOP at \(I_1\) and \(I_2\) respectively (mm Hg), and the overall resistance to outflow was determined using the formula \((R) = 1/C\). The second IOP level was maintained to perform the surgery.

**Surgical intervention**

We have described the procedure in detail in earlier reports. In summary, the surgical intervention (5x5 mm scleral flap) was performed on the eye by the same surgeon (TS). Using a crescent shaped knife a 4x4 mm deep sclero-keratectomy was performed. Schlemm’s canal was then “unroofed” and the corneal stroma excised anteriorly down to Descemet’s membrane. After IOP decreased to a stable level, the superficial scleral flap was tightly closed with 6 “X” shaped 10/0 nylon sutures. The outflow facility was assessed, first after DS and then after tight superficial flap suturing, as described above.

**RESULTS**

In 12 pig eyes, the outflow facility which was 0.164 (SD 0.014) µl/min/mm Hg preoperatively increased significantly (1.584 (SD 0.217) µl/min/mm Hg, p<0.001) after DS. When the superficial flap was closed, the aqueous outflow facility decreased significantly (0.754 (SD 0.107) µl/min/mm Hg, p<0.001) after tight superficial flap suturing.

**Abbreviations:** DS, deep sclerectomy; IOP, intraocular pressure; NPGS, non-penetrating glaucoma surgery; TDM, trabeculo-Descemet’s membrane.
The mean (SD) outflow facility after NPGS (1.584 (0.217) μl/min/mm Hg) is relatively lower than previously reported mean outflow facility after trabeculectomy\(^1\) (2.957 (0.602) μl/min/mm Hg), which possibly accounts for the gradual decrease in IOP after NPGS, as opposed to the sudden drop in pressure after perforation as in the case of trabeculectomy.

According to McEwen\(^1\) a single patent hole of 12 μm is by itself sufficiently large to provide a normal facility of outflow. Based on this we would have expected very little, if any, resistance accounted for by the tight suturing of the superficial flap. Nevertheless the outflow facility significantly decreased after the tight suturing, indicating that these technical differences could play a rather unexpected significant role.

However, in spite of significant decrease of outflow facility after tight suturing, it did not reach the level before tight suturing. This could possibly be explained by leakage, either around the wound margin or at the site of needle entry into the superficial flap, although every attempt was done at the time of surgery to ensure that there was no detectable leak. Another explanation could be that the tight suturing would force the percolating aqueous either through the remaining sclera into the uveoscleral outflow, or through the two surgically created ostia of Schlemm’s canal.

**DISCUSSION**

Although the resistance to outflow, postoperatively, is probably dependent on the TDM,\(^7\) proponents of viscoscana-lostomy\(^9\) advocate tightly suturing the superficial flap in an attempt to avoid subconjunctival filtration. Whether tightly or loosely suturing the flap affects the overall outflow facility changes after NPGS has, to our knowledge, not been investigated before.

We report an in vitro mean preoperative outflow facility in isolated porcine eyes, in line with previously published work.\(^1\) The mean (SD) outflow facility after NPGS (1.584 (0.217) μl/min/mm Hg) is relatively lower than previously reported mean outflow facility after trabeculectomy\(^1\) (2.957 (0.602) μl/min/mm Hg), which possibly accounts for the gradual decrease in IOP after NPGS, as opposed to the sudden drop in pressure after perforation as in the case of trabeculectomy.

Based on our results, the influence of the tightness of superficial flap closure on the overall resistance to outflow is by itself a significant factor in the regulation of IOP postoperatively, at least in the early postoperative period. However in the longer term there are further implications. Tightly suturing the flap would potentially influence the amount of aqueous reaching the operated site. There have been some reports\(^3\) on the inhibitory effect of aqueous humour on subconjunctival fibroblast growth, and limiting the volume and rate of aqueous passage subconjunctivally could potentially influence long term results. It is clear that further research is warranted to test this hypothesis.

One trabeculectomy study reports on the comparison between tight versus loose scleral flap closure.\(^3\) There were no obvious benefits in using a tight versus loose closure when performing trabeculectomy three months postoperatively. Nevertheless more than 60% of the tight closure group had laser suture lysis, in addition to the fact that the study does not report on medium or long term results.

Our study was conducted in vitro, but because the perfusion rate of an enucleated eye is very near that of the perfusion in vivo,\(^8\) it is reasonable to infer that we would expect very similar in vivo behaviour.

One of the main disadvantages of NPGS is its reported long learning curve.\(^6\) The NPGS model set up that we describe could be efficiently used for training ophthalmologists, thus potentially shortening this learning curve. This model differs from a regular wet lab setting in that the trainee can directly and accurately monitor the IOP changes during different steps of surgery, and thus can evaluate the accuracy of his dissection based on the pattern of IOP reduction that has been described and documented in earlier work.

It is obvious that we are still far from properly understanding the mechanisms of function of non-penetrating glaucoma surgery. Proper investigation of this could further our knowledge of the pathophysiological process of aqueous outflow, and would potentially act as a vehicle for more innovative methods—of which we are in great need.

**Authors’ affiliations**

T Shaarawy, Memorial Research Institute of Ophthalmology, Giza, Egypt

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**Figure 1** Scheme of the experimental set up. (1) 27 gauge needle catheter; (2) microsyringe pump; (3) pressure monitor; (4) chart recorder.

**Figure 2** Influence of deep sclerectomy and flap closure on the outflow facility of pig eye. After deep sclerectomy, the outflow facility was significantly increased. When the superficial scleral flap was closed, the outflow facility decreased but remained significantly higher than preoperatively. *p*<0.05, **p**<0.01, ***p***<0.001 (v preoperatively); **p**<0.01 (v after deep sclerectomy).

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