Surgical Technique

A modified model of glaucoma filtering surgery in Sprague-Dawley rats

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Animal models are useful in glaucoma research to study tissue response to wound healing. Smaller animals such as rats offer additional advantages in terms of availability of detection antibodies and microarrays with cheaper maintenance costs. In this study, we describe a glaucoma filtering surgery (GFS) model in adult Sprague–Dawley rats by performing a sclerostomy using a 26-G needle and additionally placing a silicone tube (27 G) connecting the anterior chamber to the subconjunctival space to maintain a patent fistula for the flow of aqueous humor, thus providing a more definitive bleb. This technique will be useful in identifying and modifying newer targets in the wound healing process in order to improve surgical outcomes following GFS.

Key words: Animal model, filtering bleb, glaucoma filtering surgery, sprague-dawley rats, wound healing

Animal models are often used in glaucoma research to study the healing response following glaucoma surgery. They are required especially when tissue samples are required at multiple time points, which may not be possible in humans or in cell culture. Various models of glaucoma filtering surgery (GFS) have been described for rabbits, dogs, cats, and other primates,[1-3] each having its own pros and cons. Rabbits have been considered the standard GFS model for studying wound healing and testing newer therapies due to the advantages of large eye size and the ability to carry out procedures done on humans.[4] However, they are expensive to maintain and the results cannot be directly extrapolated to human beings. The advantages of using smaller animals such as rat and mouse models of GFS include easy availability of detection antibodies and microarrays, knowledge about their genetic makeup, ability to produce genetically engineered knock-out species, and feasible logistic aspects such as easy availability and cheaper maintenance cost. Adult Sprague–Dawley (SD) rat model particularly shows less aggressive healing as compared to other existing models and has a greater resemblance to human GFS.[5] It also provides a filtering bleb with a longer bleb survival to study progressive failure as compared to other animal models, making it a promising model for glaucoma research. We describe a successful GFS model on adult SD rats to study different stages of wound healing following GFS.

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The surgery was performed on 43 SD adult rats housed in the Small Animal Research Facility, PGIMER after obtaining ethical clearance from the institutional ethical committee and institutional animal ethics committee. Healthy SD rats of either gender weighing over 150 g were shifted to the experimental area in an individual housing from the breeding area in a clean cage. They were acclimatized to the new surroundings for a day before performing any surgery. Surgical anesthesia was induced with a mixture of ketamine (50 mg/kg body weight) and xylazine (5 mg/kg body weight) injected intraperitoneally. Additionally, one drop of proparacaine 1% was instilled before performing the surgery. Postoperatively, bleb morphology anterior chamber depth and other details were assessed with the help of a stereoscopic operating microscope.

Following induction of anesthesia, the rat was placed on a sterile surgical sheet in a semi-left lateral position. Traction suture was applied to the upper eyelid using 8-0 vicryl (Ethicon Inc, Somerville NJ) [Fig. 1a], and the inferior lid was pushed under the globe to prolapse the eyeball and expose the surgical site (superior limbus and conjunctiva) [Fig. 1b]. A limbal-based conjunctival incision was made in the bulbar conjunctiva 2 mm posterior to the superior limbus [Fig. 1c] and tissue was dissected using micro scissors to raise a...
Figure 1: Various steps of the rat model of glaucoma filtering surgery. (a and b) Representative display of SD rat eye with surgical site exposed (black arrow shows the limbus). (c) The conjunctival incision (2 mm posterior to the superior limbus). (d) The 3 mm silicon tube along with pre-fixed suture. (e) Making a partial thickness scleral tunnel with a 26-G needle. (f) The aqueous egress while withdrawing the needle (arrow) (g) Insertion of a tube into the AC. (h) A filtering bleb at the end of the surgery (arrow)
limbal-based conjunctival flap. Green-colored silicone tube of 25-G soft tip (Alcon, Fort Worth, TX, USA) cannula was cut into 3-mm-long segments, and one end of the tube was cut in a bevel shape [Fig. 1d [inset]]. A 10-0 nylon suture (Aurolab, Madurai India) was applied at the junction of two-third and one-third of the tube carefully without occluding the lumen [Fig. 1d]. The same suture was then passed partial thickness through the sclera. Anterior chamber (AC) was entered using a 26-G needle by creating a needle track [Fig. 1e]. After withdrawing the needle, aqueous humor egress was visualized [Fig. 1f; arrow] and the beveled end of the silicone tube was inserted through the needle track to create a fistula between the AC and subconjunctival space. The tube was then secured to the sclera with the same pre-fixed suture [Fig. 1g]. The Tenon’s capsule was wrapped over the tube and the conjunctiva was then sutured back with one or two interrupted 10-0 nylon sutures. A filtration bleb was noted immediately at the end of surgery [Fig. 1h; dotted arrow].

Antibiotic eye drop (moxifloxacin 0.5%) was instilled at the end of the surgery. During the immediate postoperative period, the rats were observed constantly until they fully recovered from anesthesia. Rats were returned to the individual housing and provided with food and water once they maintained normal posture.

The procedure (as shown in video supplementary file 1) was completed successfully in all eyes. Most of the rats showed a good response to anesthesia after the first dose. An additional half dose of anesthesia was given in six rats. There was no delayed recovery from anesthesia or any anesthesia-related mortality. Intraoperatively, hyphema occurred due to trauma to the iris tissue by the needle in one rat eye. Further, a 26-G needle had to be passed twice in two rats for proper placement of the tube. A filtration bleb was visualized immediately post surgery in all the eyes. There was no unusual redness, watering, or any signs of post-operative infection noted in any of the rat eyes. These rats were sacrificed at different stages from day 1 to day 21 (day 1, 2, 3, 5, 7, 14, and 21) to harvest the bleb tissue for further analysis. The bleb could be identified in all the rats at the time of sacrifice. An elevated bleb with a few dilated blood vessels could be identified at all time points of sacrifice with a normal-looking anterior chamber. We did not measure the intraocular pressure (IOP) or bleb area. Tube exposure was noted at the distal end in one rat and blood clot at the proximal end of the tube in one rat in the postoperative period. Loss of tube was not noted in any of the rats during the postoperative period.

**Discussion**

We established a successful GFS model on adult SD rats by performing a sclerostomy using a 26-G needle and maintaining a patent fistula for the flow of aqueous humor by placing a silicone tube (27 G) connecting the anterior chamber to the subconjunctival space. Additionally, in our model, we also used an anchoring 10-0 nylon suture to fix the silicone tube to the scleral bed to avoid any inadvertent slippage, which could possibly close the patent fistula and compromise the flow of aqueous into the bleb. A raised bleb was observed immediately after surgery in all experiments. Sheridan *et al.* and Sherwood *et al.* established rat model of GFS on Lewis rat and Sprague–Dawley rat by using 30-G and 29-G needles, respectively, to create a fistula. In addition, to maintaining the patency for a longer period, Sherwood *et al.* used a 30-G silicon cannula. The bleb in their model survived till day 8–13. In our experimental model, we used a wider cannula (27 G) and observed an elevated bleb with some dilated vessels even up to day 21 postoperatively.

We believe this model will help the researchers establish longer-lasting filtering blebs. It also ensures that the fistula remains patent with a continued flow of aqueous in the bleb area. This is critical in experiments aimed at studying the impact of aqueous and its flow on wound healing following GFS. It will be useful in identifying and modifying newer targets in the wound healing process in order to improve surgical outcomes following GFS.

**Conclusion**

We demonstrate a simple modified technique, which includes a glaucoma filtration surgery model in adult SD rat by performing a sclerostomy using 26-G needles and placing a silicone tube (27 G) connecting the anterior chamber to the subconjunctival space to maintain a patent fistula for the flow of aqueous humor, and thus providing a more definitive bleb.

**Ethical approval**

The study was approved by the institute’s animal ethics committee (IAEC): Vide. No: 83/IAEC/537

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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