Modulating Wound Healing After Glaucoma Surgery

Advances in techniques and treatments.

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Evidence from large, long-term clinical trials now supports that IOPs in the low teens best preserve vision in patients with glaucoma.[1,2] The main determinant of long-term IOP after glaucoma filtration surgery is the healing response. Although antimetabolites have revolutionized glaucoma surgery (particularly in patient groups with a high risk of surgical failure due to scarring), the use of these agents is still associated with blinding complications.[3] Moreover, because surgery can fail despite the use of powerful antimetabolites, there is a need for better agents with improved risk-benefit ratios.

Controlling the scarring process could theoretically deliver postoperative IOPs around 10 mm Hg in all patients. Many methods now exist for modulating the numerous, complex, overlapping biological events that occur after surgical trauma (some of these events and potential modulating therapies are outlined in Table 1). This article reviews new techniques that have increased the safety of antimetabolites as well as recent advances such as growth-factor neutralization and molecular strategies to control tissue repair and healing. It should also be emphasized that surgical techniques are critical in conjunction with these antiscarring treatments.

TISSUE "PRIMING"

Long-term topical medical therapy may "prime" the tissues of the conjunctiva and eye to react adversely to surgery,[4,5] thus leading to poor outcomes. The degree of cellular response to long-term topical therapy may also result in higher postoperative IOPs.[6] Several mechanisms alter the fibroblast phenotype and lead to the activation of a fibrotic response.[7] We have found in our laboratory that the state of cellular activation can dramatically reduce the response to antimetabolites such as 5-fluorouracil (5-FU) and mitomycin C (MMC).[8] Although antimetabolites can halt cellular growth,[9,10] these growth-arrested cells are still able to stimulate neighboring cells to scar via growth factors.[11,12] These findings help explain some of the scarring that occurs after filtration surgery despite treatment with antimetabolites—especially in focal, avascular blebs, in which a central acellular area may be surrounded by a ring of growth-arrested cells still able to stimulate fibrosis (called the *ring of steel*) (Figure 1).[13]

TISSUE DAMAGE

At present, no surgical procedure is free of the postoperative scarring response. Minimizing tissue injury is important, because tissue damage is associated with the release of vari-

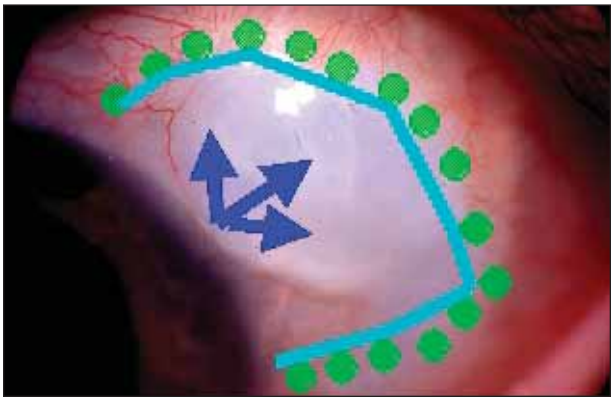

Figure 1. According to the authors' ring-of-steel hypothesis for cystic blebs, anterior aqueous drainage occurs, and a ring of scar tissue forms. Antimetabolites worsen preexisting conditions for a cystic bleb, but they are not necessary for a cystic bleb if the other conditions meeting the hypothesis are met.

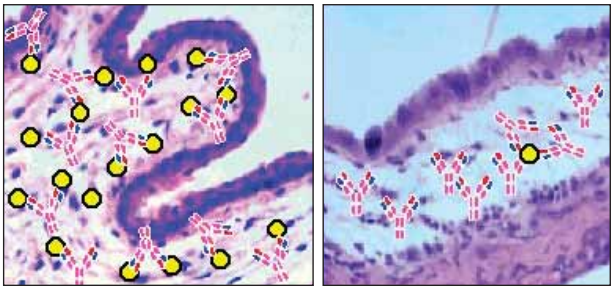

Figure 2. The antibody (represented diagrammatically in the bleb) only works if TGF-beta2 is present in the wound. For that reason, the incidence of side effects such as hypotony theoretically should be minimal, as indicated by clinical studies demonstrating a lower mean IOP without an increase in cases of very low pressures (< 9 mm Hg).[14]

ous cytokines.[15] Reducing bleeding in particular is stimulatory to healing. Another important component of ocular injury is a breakdown in the blood aqueous barrier that may not be clinically visible but may contribute to scarring and surgical failure. The growth factors released (eg, transforming growth factor beta [TGF-beta][16]) may contribute to cataract formation.[17]

INFLAMMATORY REACTION

Clinically, persistent conjunctival inflammation is associated with a pronounced scarring response.[15] Conjunctival epithelial cells may continue to express surface antigens several months after surgery, and this action may increase the ability of these cells to induce immune inflammation and fibrosis.[18] Subclinical anterior chamber inflammation such as occurs during combined cataract and glaucoma surgery has a worse prognosis than that which occurs from glaucoma surgery alone. Laser flare readings are persistently higher after cataract surgery compared with trabeculectomy, even in clinically quiet eyes. This difference helps to explain the effect of cataract surgery on healing.[17] Cataract surgery can raise the pressure in eyes that are hypotonous after glaucoma filtration surgery involving MMC use.[19] This phenomenon has been confirmed clinically by cases in which topical corticosteroid treatment after trabeculectomy has been associated with a significant reduction in final IOP.[20] The effect of nonsteroidal anti-inflammatory drugs (NSAIDs) is still uncertain.[21] Newer agents affecting aspects of the inflammation pathway include cyclosporine and cyclo-oxygenase-2 inhibitors.

BLOOD CLOTTING

Bleeding leads to clotting and fibrin formation, both of which may result in surgical failure by blocking aqueous outflow, sequestering growth factors, and stimulating scarring. Fibrinolytic agents such as tissue plasminogen activator and urokinase have been used to lyse blood clots after surgery.[22] In the short term, the use of fibrinolytic agents may lower IOP, but there is a risk of further extra- and intraocular hemorrhage.[23] In addition, the breakdown molecules (produced by lysing) may have a long-term stimulatory effect on wound healing that may result in surgical failure.[24] The prevention of clotting with agents such as heparin may be a more promising avenue for wound modulation. We demonstrated that clinical proliferative vitreoretinopathy can be prevented by a regimen combining intraoperative 5-FU and heparin.[25,26]

STIMULATORY FACTORS

A large number of small protein molecules that stimulate cells flow in the aqueous through the bleb. These molecules are known as *growth factors* or *cytokines*.[15,27] One of these, TGF-beta, stimulates more activity than the other growth factors found in the aqueous.[28] It may also be the most important growth factor. This cytokine stimulates further production of itself through autoinductive action,[29] and it may even neutralize the effect of MMC in vivo.[28,30,31] Together, these occurrences will lead to further healing.

Several agents may affect the activity of growth factors. Tranilast ((N-(3',4'-dimethoxycinnamoyl)anthranilic acid) inhibits TGF-beta activity and has antiscarring effects in the body and the eye. In experimental models, this agent has effectively inhibited scarring in experimental proliferative vitreoretinopathy[32] and PRK.[33] It also decreases conjunctival scarring after glaucoma filtration surgery. Genistein[34] and Suramin also suppress TGF-beta activity. The latter reduced postoperative scarring in an experimental model

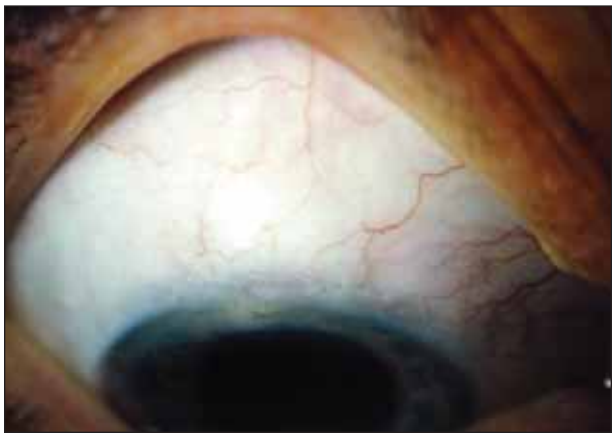

Figure 3. After treatment with the TGF-beta2 antibody (Trabio), this patient's bleb was diffuse and noncystic. Studies have indicated that prolonged injections of the antibody may extend the length of bleb survival and that they are superior to injections of 5-FU.[35]

of filtration surgery[36] and showed promise in an early pilot study.[37] Interferon-alpha, an antifibrotic cytokine, has been shown to reduce the scarring activity of fibroblasts, although a clinical trial did not demonstrate this agent to be significantly better than current antimetabolites.[38]

Our group has employed different methods of targeting TGF-beta activity, including the use of a novel antibody and antisense oligonucleotides. (8)Trabio R) (Cambridge Antibody Technology, Cambridge, UK) is a fully human monoclonal antibody specific to the active form of human TGF-beta2, which is the predominant form of TGF-beta in the aqueous. Unlike antimetabolites, one of this antibody's theoretical advantages is that it only acts if TGF-beta2 is present in the wound (Figure 2). In an animal model of aggressive conjunctival scarring, the antibody significantly improved the outcome of glaucoma filtration surgery compared to the control.[30] Compared histologically with the effects of MMC treatment, the antibody appeared to be much less destructive to local tissue.

This antibody is now being tested for clinical use. At Moorfields Eye Hospital and the Western Eye Hospital in London, a pilot clinical study of this antibody in glaucoma filtration surgery has shown no significant side effects or inflammatory reaction. Investigators have also reported an IOP reduction of 4.6 mm Hg in the placebo group versus 10.4 mm Hg in the antibody-treated group at 1 year; the latter group also required fewer interventions[39] and lacked the cystic blebs usually associated with antimetabolite use. A comparable trial of the antibody in phacotrabeculectomy demonstrated similarly encouraging results, and several multicenter studies are currently underway (Figure 3).

FIBROBLAST ACTIVITY

MMC and 5-FU

The anticancer agents MMC and 5-FU are the main antiscarring agents presently used, although some trials still involve single applications of beta-radiation, which has the advantage of good bleb morphology.[40] Based on the work of Parrish et al,[41] subconjunctival 5-FU became the first established antimetabolite regimen. Since then, the intraoperative regimens for MMC[42] and 5-FU that we introduced (based on our cell culture results showing that short applications of antimetabolites could arrest long-term cellular growth[9,10,43-45]) have become much more popular due to their convenient use at the time of surgery, supplemented by 5-FU injections. A recent meta-analysis, however, suggested that fewer than four injections of 5-FU may have little effect.[46] Both MMC and 5-FU treatments are generally associated with relatively small treatment areas, combined with limbus-based surgery and a posterior incision. They have resulted in thin, avascular blebs and complications including leakage, hypotony, and endophthalmitis on long-term follow-up.[3] Methods to treat these thin, leaking blebs (eg, compression sutures and blood[47] or Nd:YAG-induced subconjunctival bleeding[48]) are not totally satisfactory. An interpalpebral or inferiorly placed bleb is associated with a very high incidence of complications (up to 10 times the normal rate).

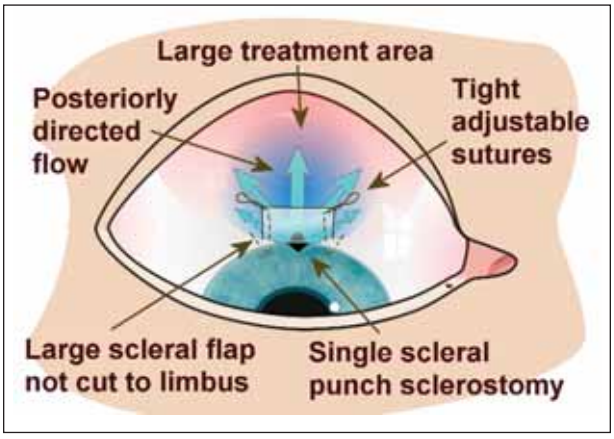

Figure 4. Simple changes minimize the occurrence of complications after trabeculectomy, particularly when used in conjunction with strong antimetabolites such as MMC.

Optimizing the Effects of Antimetabolites

Techniques to minimize the incidence of complications associated with antimetabolite use in glaucoma filtration surgery include optimizing the choice of agent, method of its application, and surgical technique (Figure 4). For example, one can use different single-application antiscarring agents and titrate concentrations against patient risk factors. This method is particularly important if the patient has a lower risk of scarring but still requires low target pressures, such as in normal-tension glaucoma. A study from Moorfields Eye Hospital showed that, although intraoperative MMC lowered IOP more than intraoperative 5-FU, the former agent produced many more complications, thus illustrating the importance of choosing the right agent and dose[49] (Table 2).

Size of Antimetabolite Treatment Area

In our ring-of-steel hypothesis, we proposed that a ring of scar tissue forms that restricts the bleb, thus making it focal and thin. Based on this hypothesis, we have shown that antimetabolite treatment area greatly influences bleb morphology; small treatment areas give rise to thin-walled and cystic blebs, whereas large treatment areas are associated with more diffuse-looking, thicker, walled blebs. Our simple clinical observation of cystic blebs revealed two common features: (1) anterior aqueous drainage at the

TABLE 1. SEQUENCE OF EVENTS IN WOUND HEALING AND POSSIBLE METHODS OF MODULATION AFTER GLAUCOMA FILTERING SURGERY*†

Event	Possible Modulation
Activated conjunctiva with "pre-activated" cells	Stop medical therapy (especially drops causing red eye) Preoperative steroids[50]
Conjunctival/episcleral/scleral incisions Damage to connective tissue	Minimize trauma by using less invasive surgical techniques
Release of plasma proteins and blood cells	Hemostasis (blood can reverse MMC)
Activation of clotting and complement fibrin/fibronectin/blood cell clot	Agents preventing/removing fibrin (eg, heparin, tissue plasminogen activator, hirudin)[25]
Release of growth factors from blood	Antagonists to growth factor production (eg, antibodies to growth factors humanized anti-TGF-beta2 antibody [CAT 152 Trabio] or receptors[39]) Antisense oligonucleotides, ribozymes[51] Less specific antagonists (eg, tranilast, genistein, suramin)[37]
Aqueous released from eye Breakdown of blood aqueous barrier Release of growth factors into aqueous Aqueous begins to flow through wound	Blood aqueous barrier stabilizing agents (eg, steroids) NSAIDs
Migration and proliferation of polymorphonuclear neutrophil cells, macrophages, and lymphocytes	Anti-inflammatory agents (eg, steroids, cyclosporine) Antimetabolites (eg, 5-FU or MMC) Antibodies to inflammatory mediators Angiotensin-converting enzyme or chymase inhibitors
Activation, migration, and proliferation of fibroblasts	Preoperative steroids to reduce activation Antimetabolites MMC and 5-FU Methylxanthine derivatives, mushroom lectins[52] Antiproliferative gene p21(WAF-1/Cip-1)[53] Photodynamic therapy[54]
Wound contraction	Anticontraction agents (eg, colchicine,[55] taxol lectins, MMP inhibitors[56,57])
Fibroblast synthesis of tropocollagen glycosaminoglycans and fibronectin	Interferon alpha,[58] MMP inhibitors, fibrostatin-c
Collagen cross-linking and modification	Anticross-linking agents (eg, Beta-aminopropionitrile, penicillamine)
Blood vessel endothelial migration and proliferation	Inhibitors of angiogenesis (eg, fumagillin analogs, heparin analogs)
Resolution of healing, apoptosis, and disappearance of fibroblasts	MMC, 5-FU, death receptor ligands,[59] stimulants of apoptosis pathways
Fibrous subconjunctival scar	

**Adapted and reprinted with permission from Khaw et al.[28]*
†Events and agents have overlapping time duration and action.

Figure 5. This patient underwent a limbus-based trabeculectomy with a small area of MMC on the left. The bleb was small and cystic, and he experienced discomfort and recurrent attacks of blebitis. His right eye had a fornix-based trabeculectomy with a large area of MMC treatment. This eye's diffuse, noncystic bleb was comfortable and not associated with any complications over 7 years.

limbus and (2) a ring of scar tissue or the ring of steel.[60] Our hypothesis has subsequently been confirmed experimentally[61] and clinically.[62,63] In 1996, our emphasis on creating large MMC treatment areas to yield diffuse, noncystic blebs and our use of fornix-based flaps dramatically lowered our rate of severe bleb-related complications over 3 to 5 years from 20% to zero (Figure 5).

Application Technique

Intraoperatively applying antimetabolites under the scleral flaps as well as under the conjunctiva appears to be advantageous.[64] Applying the agents prior to entering the anterior chamber seems to avoid ciliary body toxicity. The time of antimetabolite exposure varies among surgeons and centers. A recent study showed that subconjunctival uptake rises sharply, then peaks at approximately 3 minutes, with only a small rise after that.[65] Because varying the concentration appears to be a more accurate way of determining tissue uptake than changing the agent's volume,[66] we try to fix exposure times at a minimum of 3 minutes and vary the agent and its concentration for different risk groups. Table 3 shows the current Moorfields regimen.

The advent of surgical techniques such as the use of releasable sutures has permitted better regulation of pressure control, but they are still associated with hypotony some months after surgery. A new adjustable suture with special forceps (2-502; Duckworth & Kent Ltd., Hertfordshire, England) allows the eye's IOP to be gradually and gently decreased toward target level (Figure 6).[13,67,68]

Other Antifibroblastic Therapies

Photodynamic therapy with diffuse blue light coupled with a photosensitizing agent to kill fibroblasts may be another way to control the surface area of treatment and modulate healing.[69] It will be important to determine the effect of these agents on the overlying epithelium, because differentiated stable epithelium may have a suppressive effect on fibroblasts in the wound.[70,71] A pilot clinical trial has yielded promising results.[54] Other approaches to controlling proliferation include agents such as mushroom lectins[52] and methylxanthines[72] and overexpressing genes inhibiting proliferation such as p21 WAF-1/CIP-1 introduced via an adenovirus system[11] but not producing the bleb thinning caused by MMC, antagonising integrins and their receptors,[73] or altering intracellular transcription.[74]

CELL MIGRATION, TISSUE CONTRACTION, AND EXTRACELLULAR MATRIX SYNTHESIS

Fibroblast cell migration is an essential component of tissue contraction, which is important in filtration surgery failure. The matrix metalloproteinases (MMPs) are enzymes that degrade the extracellular matrix. The levels of MMPs in the vitreous relate to the development of retinal scarring,[75] and, importantly, they can inhibit cell-mediated collagen contraction. This ability applies to the retinal pigment epithelium[76] as well as Tenon's fibroblasts.[56] We have now demonstrated dramatically reduced scarring in an experimental model, with retention of normal tissue morphology using an MMP inhibitor. The action is equivalent to that of MMC but without the deleterious side effects.[56] A number of agents (including taxol and etoposide [microtubule-stabilizing agents] and cytochalasin B [a microfilament inhibitor]) can affect the cytoskeleton of the cell and hence inhibit migration. Taxol and etoposide have been used in models of filtration surgery to prolong bleb survival,[77] although neither has been assessed in human clinical trials to date.

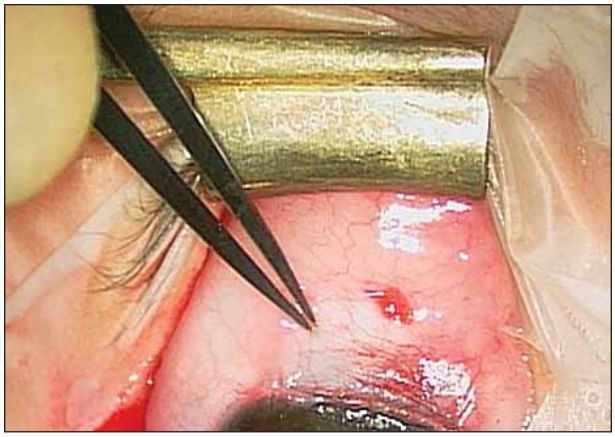

Figure 6. A surgeon uses a special pair of smooth, noncutting forceps to perform transconjunctival adjustment of adjustable sutures. The IOP can be lowered gradually, thus minimizing the chance of hypotony.

TABLE 2. RISK FACTORS FOR FAILURE DUE TO SCARRING AFTER GLAUCOMA FILTRATION SURGERY*

Risk Factors	Risk (+ to +++)	Comments
1) OCULAR		
Neovascular glaucoma (active)	+ + +	
Previous failed filtration surgery	+ + (+)	
Previous conjunctival surgery	+ +	Uncertain
Chronic conjunctival inflammation	+ + (+)	
Previous cataract extraction (conjuctival incision)	+ + (+)	
Aphakia (intracapsular extraction)	+ + +	
Previous intraocular surgery	+ +	Depends on type of surgery
Uveitis (active, persistent)	+ +	
A red, injected eye	+ +	
Previous topical medications (beta-blockers + pilocarpine) (beta-blockers + pilocarpine + adrenaline) New topical medications	+ (+) + + + + (+)	Particularly if they cause a red eye
High preoperative IOP (higher with each 10-mm Hg rise)	+ (+)	May be associated with higher levels of growth factor
Time since last surgery (especially if within last 30 days)	+ + + (+)	
Inferiorly located trabeculectomy	+	Possibly increased exposure and inflammation
2) PATIENT		
Afro-Caribbean origin, may vary (eg, West vs East Africans)	+ + + + (+) +	
Indian subcontinent origin	+	
Hispanic origin	(+)	
Japanese origin	(+)	
Elderly Young Children	(+) +(+) ++	

**In this table, + indicates the lowest risk, +++ represents the highest risk, and (+) signifies possible risk.*

MATRIX SYNTHESIS

Some agents primarily interfere with molecular crosslinking of collagen, such as ß-aminopropionitrile and D-penicillamine. Experimental and clinical evidence suggest that these agents may work in filtration surgery.[78] Fibrostatin-c, an inhibitor of prolyl-4-hydroxylase, has been shown to inhibit type I collagen secretion by Tenon's capsule fibroblasts.[79] Methylxanthine derivatives also reduce collagen secretion.[72] Interestingly, MMP inhibition also reduces collagen synthesis in vitro,[56] which may help to explain the dramatic reduction in scar tissue in vivo after MMP use.[57]

APOPTOSIS

Relatively little is known about the physiologic signals initiating the end of wound healing, although fibroblast apoptosis is probably an important component.[80] MMC and high doses of 5-FU not only inhibit fibroblast proliferation and cause long-term growth arrest, but they also induce apoptosis.[81] The fibroblasts not killed but arrested in their growth by MMC can prevent inflammatory T-lymphocytes from apoptosing, thus resulting in persistent inflammation.[59,82] Another strategy of inducing apoptosis involves proapoptotic peptides. The induction and regulation of apoptotic mechanisms may offer a novel way of regulating and terminating excessive and unwanted healing.

CONCLUSION

Surgeons' ability to fully modulate the healing process would ultimately allow them to determine the long-term, final IOP in patients undergoing glaucoma filtration surgery. Increasing understanding of the healing and repair processes as well as developments in modern scientific

TABLE 3. MOORFIELDS EYE HOSPITAL'S INTRAOPERATIVE, SINGLE-DOSE, ANTISCARRING REGIMEN*†

Patient Category	Characteristics	Regimen
Low-Risk	No risk factors Topical medications (eg, beta-blockers, pilocarpine) Afro-Caribbean origin and elderly <40 years of age with no other risk factors	Nothing or intraoperative 5-FU 50 mg/mL††§ for 5 minutes, as per the Moorfields "More Flow" study
Intermediate-Risk	Topical medications (eg, adrenaline) Previous cataract surgery without conjunctival incision (capsule intact) Several low-risk factors Combined glaucoma filtration surgery/cataract extraction Previous conjunctival surgery (eg, squint surgery, retinal detachment surgery, trabeculotomy)	Intraoperative 5-FU 50 mg/mL†† for 5 minutes or MMC 0.2 mg/mL§ for 3 minutes, as per the Moorfields "More Flow" study
High-Risk	Neovascular glaucoma Chronic, persistent uveitis Previous failed trabeculectomy/tubes Chronic conjunctival inflammation Multiple risk factors Aphakic glaucoma	Intraoperative MMC 0.5 mg/mL§ for 3 minutes (a tube may be more appropriate in many of these cases, combined with MMC)

**Circa 2004. Regimen is continuously evolving.*
†Lower target pressures likely require stronger agents.
††Intraoperative beta-radiation 1,000 cGy can also be used. In the future, CAT-152 (Trabio) or humanized anti-TGF-beta2 antibody may be appropriate in the low- and intermediate-risk groups based on the results of current studies. These groups account for the majority of patients undergoing glaucoma surgery.
§Postoperative 5-FU injections can be given in addition to the intraoperative applications of antimetabolites.

techniques in cell and molecular biology will ultimately lead to better, safer therapies for all patients. ❑

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1. AGIS Investigators. The Advanced Glaucoma Intervention study (AGIS) 7. The relationship between control of intraocular pressure and visual field deterioration. *Am J Ophthalmol.* 2000;130:429-440.
2. Leske MC, Heijl A, Hussein M, et al. Factors for glaucoma progression and the effect of treatment: the early manifest glaucoma trial. *Arch Ophthalmol.* 2003;121:48-56.
3. Rothman RF, Liebmann JM, Ritch R. Low-dose 5-fluorouracil trabeculectomy as initial surgery in uncomplicated glaucoma: long-term followup. *Ophthalmology.* 2000;107:1184-1190.
4. Broadway DC, Grierson I, O'Brien C, Hitchings RA. Adverse effects of topical anti-glaucomatous medication regimens: Part I. Effect on the cell profile of the conjunctiva. *Arch Ophthalmol.* 1994;112:1437-1445.
5. Broadway DC, Grierson I, O'Brien C, Hitchings RA. Adverse effects of topical antiglaucoma medication: Part II. The outcome of filtration surgery. *Arch Ophthalmol.* 1994;112:1446-1454.
6. Arici MK, Demircan S, Topalkara A, et al. Effect of conjunctival structure and inflammatory cell counts on intraocular pressure after trabeculectomy. *Ophthalmologica.* 1999;213:371-375.
7. Fini ME. Keratocyte and fibroblast phenotypes in the repairing cornea. *Prog Retin Eye Res.* 1999;18:529-551.
8. Occleston NL, Alexander RA, Mazure A, et al. Effects of single exposures to antiproliferative agents on ocular fibroblast-mediated collagen contraction. *Invest Ophthalmol Vis Sci.* 1994;35:3681-3690.
9. Khaw PT, Sherwood MB, MacKay SLD, et al. 5-minute treatments with fluorouracil, floxuridine and mitomycin have long-term effects on human Tenon's capsule fibroblasts. *Arch Ophthalmol.* 1992;110:1150-1154.
10. Khaw PT, Doyle JW, Sherwood MB, et al. Prolonged localized tissue effects from 5-minute exposures to fluorouracil and mitomycin C. *Arch Ophthalmol.* 1993;111:263-267.
11. Occleston NL, Daniels JT, Tarnuzzer RW, et al. Single exposures to antiproliferatives: long-term effects on ocular fibroblast wound-healing behavior. *Invest Ophthalmol Vis Sci.* 1997;38:1998-2007.
12. Daniels JT, Occleston NL, Crowston JG, Khaw PT. Effects of antimetabolite induced cellular growth arrest on fibroblast-fibroblast interactions. *Exp Eye Res.* 1999;69:117-127.
13. Khaw PT, Chang LPY. Antifibrotic agents in glaucoma surgery. In: Duker D, Yanoff.M., eds. *Ophthalmology—A Practical Textbook.* London: Churchill Livingston; 2003: 1596-1603.
14. Clarke JCK, Broadway DC, Migdal CS, et al. Longer term follow-up of adjunctive anti-TGFbeta2

human monoclonal antibody as an agent to prevent scarring following phacotrabeculectomy. Paper presented at: The ARVO Annual Meeting; April 25, 2004; For Lauderdale, FL.
15. Chang L, Crowston JG, Cordeiro MF, et al. The role of the immune system in conjunctival wound healing after glaucoma surgery. *Surv Ophthalmol.* 2000;45:49-68.
16. Lovicu FJ, Schulz MW, Hales AM, et al. TGF beta induces morphological and molecular changes similar to human anterior subcapsular cataract. *Br J Ophthalmol.* 2002;86:220-226.
17. Siriwardena D, Kotecha A, Minassian D, et al. Anterior chamber flare after trabeculectomy and after phacoemulsification. *Br J Ophthalmol.* 2000;84:1056-1057.
18. Ihan A, Cvenkel B. Conjunctival epithelium expression of HLA-DR in glaucoma patients and its influence on the outcome of filtration surgery. *Br J Ophthalmol.* 2000; 84(6):648-650.
19. Doyle JW, Smith MF. Effect of phacoemulsification surgery on hypotony following trabeculectomy surgery. *Arch Ophthalmol.* 2000;118:763-765.
20. Araujo SV, Spaeth GL, Roth SM, Starita RJ. A ten-year follow-up on a prospective, randomized trial of postoperative corticosteroids after trabeculectomy. *Ophthalmology.* 1995;102:1753-1759.
21. Migdal C, Hitchings R. The developing bleb: Effect of topical antiprostaglandins on the outcome of glaucoma fistulizing surgery. *Br J Ophthalmol.* 1983;67:655-660.
22. WuDunn D. Intracameral urokinase for dissolution of fibrin or blood clots after glaucoma surgery. *Am J Ophthalmol.* 1997;124:693-695.
23. Azuara-Blanco A, Wilson RP. Intraocular and extraocular bleeding after intracameral injection of tissue plasminogen activator. *Br J Ophthalmol.* 1998;82:1345-1346.
24. Gray AJ, Bishop JE, Reeves JT, Laurent GJ. A alpha and B beta chains of fibrinogen stimulate proliferation of human fibroblasts. *J Cell Sci.* 1993;104(Pt 2):409-413.
25. Asaria RH, Kon CH, Bunce C, et al. Adjuvant 5-fluorouracil and heparin prevents proliferative vitreoretinopathy: Results from a randomized, double-blind, controlled clinical trial. *Ophthalmology.* 2001;108:1179-1183.
26. Kon CH, Occleston NL, Foss A, et al. Effects of single, short-term exposures of human retinal pigment epithelial cells to thiotepa or 5-fluorouracil: implications for the treatment of proliferative vitreoretinopathy. *Br J Ophthalmol.* 1998;82:554-560.
27. Tripathi RC, Borisuth NSC, Tripathi BJ. Growth factors in the aqueous humour, and their therapeutic implications in glaucoma and anterior segments disorders of the human eye. *Drug Dev Res.* 1991;22:1-23.
28. Khaw PT, Occleston NL, Schultz GS, et al. Activation and suppression of fibroblast activity. *Eye.* 1994;8:188-195.
29. Denk PO, Roth-Eichhorn S, Gressner AM, Knorr M. Effect of cytokines on regulation of the production of transforming growth factor beta-1 in cultured human Tenon's capsule fibroblasts. *Eur J Ophthalmol.* 2000;10:110-115.
30. Cordeiro MF, Gay JA, Khaw PT. Human anti-transforming growth factor-beta2 antibody: a new glaucoma anti-scarring agent. *Invest Ophthalmol Vis Sci.* 1999;40:2225-2234.
31. Doyle JW, Smith MF, Garcia JA, , et al. Treatment of bleb leaks with Transforming Growth Factor-B in the rabbit model. *Invest Ophthalmol Vis Sci.* 1997;38:1630-1634.
32. Ito S, Sakamoto T, Tahara Y, et al. The effect of tranilast on experimental proliferative vitreoretinopathy. *Graefes Arch Clin Exp Ophthalmol.* 1999;237:691-696.
33. Furukawa H, Nakayasu K, Gotoh T, et al. Effect of topical tranilast and corticosteroids on subepithelial haze after photorefractive keratectomy in rabbits. *J Refract Surg.* 1997; 13(5 suppl):457-458.
34. Kim H, Peterson TG, Barnes S. Mechanisms of action of the soy isoflavone genistein: emerging role of its effects via transforming growth factor beta signaling pathways. *Am J Clin Nutrition.* 1998;68(suppl):1418-1425.
35. Mead AL, Wong TTL, Cordiero MF, et al. Anti-Transforming Growth Factor–b2 antibody: a new postoperative anti-scarring agent in glaucoma surgery. *Invest Ophthalmol Vis Sci.* 2003;44:3394-3401.
36. Mietz H, Chevez-Barrios P, Feldman RM, Lieberman MW. Suramin inhibits wound healing following filtering procedures for glaucoma. *Br J Ophthalmol.* 1998;82:816-820.
37. Mietz H, Krieglstein GK. Suramin to enhance glaucoma filtering procedures: a clinical comparison with mitomycin. *Ophthalmic Surg Lasers.* 2001;32:358-369.
38. Gillies MC, Brooks AMV, Young S, et al. A randomized phase II trial of interferon-alpha2b versus 5-fluorouracil after trabeculectomy. *Australian and New Zealand Journal of Ophthalmology.* 1999;27:1:37-44.
39. Siriwardena D, Khaw PT, King AJ, et al. Human antitransforming growth factor beta(2) monoclonal antibody—a new modulator of wound healing in trabeculectomy: a randomized placebo controlled clinical study. *Ophthalmology.* 2002;109:427-431.
40. Khaw PT, Ward S, Grierson I, Rice NSC. The effects of beta-radiation on proliferating human Tenon's capsule fibroblasts. *Br J Ophthalmol.* 1991;75:580-583.
41. The Fluorouracil Filtering Surgery Study Group. Fluorouracil filtering surgery study one-year follow-up. *Am J Ophthalmol.* 1989;108:625-635.
42. Chen CW. Enhanced intraocular pressure controlling effectiveness of trabeculectomy by local application of mitomycin C. *Trans Asia Pacif Acad Ophthalmol.* 1983;9:172-177.
43. Khaw PT, Doyle JW, Sherwood MB, et al. Effects of intraoperative 5-fluorouracil or mitomycin C on glaucoma filtration surgery in the rabbit. *Ophthalmology.* 1993;100:367-372.
44. Khaw PT, Ward S, Porter A, et al. The long-term effects of 5-fluorouracil and sodium butyrate on human Tenon's fibroblasts. *Invest Ophthalmol Vis Sci.* 1992;33:2043-2052.
45. Smith MF, Sherwood MB, Doyle JW, Khaw PT. Results of intraoperative 5-fluorouracil supplementation on trabeculectomy for open-angle glaucoma. *Am J Ophthalmol.* 1992;114:737-741.
46. Wormald R, Wilkins MR, Bunce C. Post-operative 5-fluorouracil for glaucoma surgery. *Cochrane Database Syst Rev.* 2000;2:CD001132.
47. Haynes WL, Alward WL. Combination of autologous blood injection and bleb compression sutures to treat hypotony maculopathy. *J Glaucoma.* 1999;8:384-387.
48. Pilcher BK, Wang M, Qin XJ, et al. Role of matrix metalloproteinases and their inhibition in cutaneous wound healing and allergic contact hypersensitivity. *Ann N Y Acad Sci.* 1999;878:12-24.
49. Membrey WL, Poinoosawmy DP, Bunce C, Hitchings RA. Glaucoma surgery with or without adjunctive antiproliferatives in normal tension glaucoma: 1 intraocular pressure control and complications. *Br J Ophthalmol.* 2000;84:586-590.
50. Broadway DC, Grierson I, Sturmer J, Hitchings RA. Reversal of topical antiglaucoma medication effects on the conjunctiva. *Arch Ophthalmol.* 1996;114:262-267.
51. Cordeiro MF, Mead A, Ali RR, et al. Novel antisense oligonucleotides targeting TGF-beta inhibit in vivo scarring and improve surgical outcome. *Gene Ther.* 2003;10:59-71.
52. Batterbury M, Tebbs CA, Rhodes JM, Grierson I. Agaricus bisporus (edible mushroom lectin) inhibits ocular fibroblast proliferation and collagen lattice contraction. *Exp Eye Res.* 2002;74:361-370.
53. Perkins TW, Faha B, Ni M, et al. Adenovirus-mediated gene therapy using human p21WAF-1/Cip-1 to prevent wound healing in a rabbit model of glaucoma filtration surgery. *Arch Ophthalmol.* 2002;120:941-949.
54. Diestelhorst M, Grisanti S. Photodynamic therapy to control fibrosis in human glaucomatous eyes after trabeculectomy: a clinical pilot study. *Arch Ophthalmol.* 2002;120:130-134.
55. Molteno ACB, Straughan JL, Ancker E. Control of bleb fibrosis after glaucoma surgery by anti-inflammatory agents. *S Afr Med J.* 1976;50:881-885.
56. Daniels JT, Cambrey AD, Occleston NL, et al. Matrix metalloproteinase inhibition modulates fibroblast-mediated matrix contraction and collagen production in vitro. *Invest Ophthalmol Vis Sci.* 2003;44:1104-1110.
57. Wong TT, Mead AL, Khaw PT. Matrix metalloproteinase inhibition modulates postoperative scarring after experimental glaucoma filtration surgery. *Invest Ophthalmol Vis Sci.* 2003;44:1097-1103.
58. Gillies M, Su T, Sarossy M, Hollows F. Interferon-alpha 2b inhibits proliferation of human Tenon's capsule fibroblasts. *Graefes Arch Clin Exp Ophthalmol.* 1993;231:118-121.
59. Crowston JG, Chang LH, Constable PH, et al. Apoptosis gene expression and death receptor signaling in mitomycin-C-treated human Tenon capsule fibroblasts. *Invest Ophthalmol Vis Sci.* 2002;43:692-699.
60. Khaw PT, Wilkins M. Antifibrotic agents in glaucoma surgery. In: Myron Yanoff, Jay S Duker, eds. *Ophthalmology.* London: Mosby International Ltd; 1998: 31.1-32.6.
61. Cordeiro MF, Constable PH, Alexander RA, et al. Effect of varying the mitomycin-C treatment area in glaucoma filtration surgery in the rabbit. *Invest Ophthalmol Vis Sci.* 1997;38:1639-1646.
62. Susanna R, Costa VP, Malta RF, et al. Intraoperative mitomycin-C without conjunctival and Tenon's capsule touch in primary trabeculectomy. *Ophthalmology.* 2001;108:1039-1042.
63. Wells AP, Bunce C, Khaw PT. Flap and suture manipulation after trabeculectomy with adjustable sutures: Titration of flow intraocular pressure in guarded filtration surgery. *J Glaucoma.* In press.
64. El Sayyad F, Belmekki M, Helal M, et al. Simultaneous subconjunctival and subscleral mitomycin C application in trabeculectomy. *Ophthalmology.* 2000;107:298-301.
65. Wilkins MR, Occleston NL, Kotecha A, et al. Sponge delivery variables and tissue levels of 5-fluorouracil. *Br J Ophthalmol.* 2000;84:92-97.
66. Vass C, Georgopoulos M, El Menyawi I, et al. Intrascleral concentration vs depth profile of mitomycin-C after episcleral application: impact of applied concentration and volume of mitomycin-C solution. *Exp Eye Res.* 2000;70:571-575.
67. Khaw PT. Advances in glaucoma surgery: evolution of antimetabolite adjunctive therapy. *J Glaucoma.* 2001;10(5 suppl 1):81-84.
68. Wells AP, Cordeiro MF, Bunce C, Khaw PT. Cystic bleb formation and related complications in limbus versus fornix based conjunctival flaps in paediatric and young adult trabeculectomy with mitomycin C. *Ophthalmology.* 2003;110:2192-2197.
69. Grisanti S, Gralla A, Maurer P, et al. Cellular photoablation to control postoperative fibrosis in filtration surgery: in vitro studies. *Exp Eye Res.* 2000;70:145-152.
70. Daniels JT, Khaw PT. Temporal stimulation of corneal fibroblast wound healing activity by differentiating epithelium in vitro. *Invest Ophthalmol Vis Sci.* 2000;41:3754-3762.
71. Wilson SE, Liu JJ, Mohan RR. Stromal-epithelial interactions in the cornea. *Prog Retin Eye Res.* 1999;18:293-309.
72. Saika S, Yamanaka O, Okada Y, et al. Pentoxifylline and pentifylline inhibit proliferation of human Tenon's capsule fibroblasts and production of type-I collagen and laminin in vitro. *Ophthalmic Res.* 1996;28:165-170.
73. Paikal D, Zhang G, Cheng Q, Lee DA. The effect of integrin antibodies on the attachment and proliferation of human Tenon's capsule fibroblasts. *Exp Eye Res.* 2000;70:393-400.
74. Akimoto M, Hangai M, Okazak K, et al. Growth inhibition of cultured human Tenon's fibroblastic cells by targeting the E2F transcription factor. *Exp Eye Res.* 1998;67:395-401.
75. Kon CH, Occleston NL, Charteris D, et al. A prospective study of matrix metalloproteinases in proliferative vitreoretinopathy. *Invest Ophthalmol Vis Sci.* 1998;39:1524-1529.
76. Sheridan CM, Occleston NL, Hiscott P, et al. Matrix metalloproteinases: a role in the contraction of vitreo-retinal scar tissue. *Am J Pathol.* 2001;159:1555-1566.
77. Jampel HD, Moon J. The effect of paclitaxel powder on glaucoma filtration surgery in rabbits. *J Glaucoma.* 1998;7:170-177.
78. McGuigan LJB, Mason RP, Sanchez R, Quigley HA. D-penicillamine and beta-aminopropionitrile effects on experimental filtering surgery. *Invest Ophthalmol Vis Sci.* 1987;28:1625-1629.
79. Saika S, Ooshima A, Yamanaka O, et al. Effect of fibrostatin C, an inhibitor of prolyl 4-hydroxylase, on collagen secretion by human Tenon's capsule fibroblasts in vitro. *Graefes Arch Clin Exp Ophthalmol.* 1996;234(suppl 1):214-222.
80. Desmouliere A, Redard M, Darby I, Gabbiani G. Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. *Am J Pathol.* 1995;146:56-66.
81. Crowston JG, Akbar AN, Constable PH, et al. Antimetabolite-induced apoptosis in Tenon's capsule fibroblasts. *Invest Ophthalmol Vis Sci.* 1998;39:449-454.
82. Chang L, Crowston JG, Sabin CA, et al. Human Tenon's fibroblast-produced ifnbeta and the prevention of t-cell apoptosis. *Invest Ophthalmol Vis Sci.* 2001;42:1531-1538.