

Injected Versus Sponge-Applied Mitomycin C (MMC) During Modified Trabeculectomy in New Zealand White Rabbit Model

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Purpose: Mitomycin C is routinely applied during trabeculectomy surgeries to enhance bleb survival after glaucoma filtration surgery. The current approach involves placing cellulose sponges soaked in mitomycin C at a standard concentration onto bare sclera for a predetermined duration, which varies among surgeons. The purpose of this study was to compare the effects of sponge-applied versus intra-Tenon injection of mitomycin C during modified trabeculectomy.

Methods: Two groups of five New Zealand White rabbits underwent glaucoma filtration surgery with either preoperative intra-Tenon injection of mitomycin C or intraoperative application of mitomycin C using a cellulose sponge. Postoperative intraocular pressure was recorded weekly, and eyes were enucleated and sent for pathological examination and histological analysis.

Results: An intra-Tenon injection of mitomycin C resulted in decreased intraocular pressure measurements and bleb vascularity compared to the controls but increased levels compared to the sponge-applied group. Collagen deposition and cellularity were reduced and the goblet cell population was increased in the intra-Tenon injection group.

Conclusions: This study shows that an intra-Tenon injection can be an effective method for administering mitomycin C compared to the standard-of-care approach of mitomycin C being sponge applied onto bare sclera. Mitomycin C injection led to a greater reduction in intraocular pressure and inhibition of fibroblasts. The associated goblet cell population that can lead to increased mitomycin C toxicity-related morbidity was minimized with the intra-Tenon injection compared to the sponge-applied MMC treatment. Therefore, patients with ocular surface disease may benefit from an intra-Tenon injection.

Translational Relevance: This project provides a direct, qualitative assessment in an animal model of common techniques within glaucoma filtration surgery for drug delivery to improve surgical success.

Introduction

Glaucoma is a chronic and progressive process resulting in an optic neuropathy with characteristic visual field deficits. In the United States, glaucoma affects over 2 million Americans and is expected to drastically increase within the coming decades due to our aging population.¹ As of 2011, 2.7 million patients in the United States suffered from primary open-angle glaucoma (POAG) alone, and the number of patients with POAG is projected to increase to over 7 million by the year 2050.¹ Trabeculectomy is a common glaucoma procedure used to facilitate lowering of intraocular pressure (IOP) and halt progression of glaucomatous nerve damage when topical medications and laser treatments have failed to produce a satisfactory clinical response. The current standard-of-care approach involves the application of mitomycin C (MMC) via cellulose sponge to bare sclera for a specified period of time, typically a few minutes, to improve survival of the bleb and the success of a trabeculectomy surgery.

MMC is an antifibrotic agent that is commonly used during trabeculectomy to limit the fibrotic response, which is primarily responsible for bleb failure. MMC, originally isolated from the bacteria *Streptomyces caespitosus*, prevents DNA synthesis by acting as an alkylating agent. MMC is an effective inhibitor of fibroblast proliferation. By affecting fibroblasts in all stages of the cell cycle, it suppresses fibrosis and vascular ingrowth; however, exposure to this medication poses certain side effects and risks. Ophthalmic complications of MMC include corneal decompensation due to damage to the endothelium, anterior chamber inflammation, scleritis, and hypotony, with resultant maculopathy and choroidal detachment.² Additionally, the use of MMC-enhanced glaucoma surgeries is associated with increased incidence of bleb avascularity, transconjunctival oozing, and delayed leaks.³

It is difficult to precisely quantify the amount of MMC delivered when the cellulose sponge technique is utilized; this is the clinical uncertainty underlying the premise of this study. In previous studies, the duration of MMC exposure was subjectively chosen based on clinical examination; however, the time of exposure of a standardized concentration of MMC has not been shown to correlate with success or comorbidity following trabeculectomy surgery.⁴

Several questions thus remain, such as the amount of time required to achieve a long-term successful outcome with trabeculectomy and the concentration of MMC required for a successful procedure. Further, exposure of ocular tissues other than the sclera and subconjunctival space including Tenon's capsule to MMC could potentially result in ocular toxicity.

Thus, an increasing practice during trabeculectomy is to inject, via an intra-Tenon approach, a standardized dose of MMC. Although anecdotal evidence exists, there has been no direct comparison. This study utilized a New Zealand White rabbit model to compare intraocular pressures and bleb morphology of sponge-applied versus intra-Tenon-injected MMC during modified trabeculectomy.

Methods

Study Design

The project involved 10 young male New Zealand White rabbits undergoing a modified trabeculectomy procedure. The rabbits weighed 2 to 3 kg and were 3 to 4 months old. All animal experiments performed adhered to the University of Pittsburgh and ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee (IACUC), and complied with the tenets of the Declaration of Helsinki.

Two groups of five animals each underwent treatment with MMC. Group 1 received MMC as an intra-Tenon injection (0.2 mL \times 0.1 mg/mL = 20 μ g total injected); group 2 received MMC applied by a cellulose sponge (concentration of 0.4 mg/mL for 4 minutes applied to bare sclera). Both the right and left eye of each rabbit underwent modified trabeculectomy, in which the right eye was treated with MMC via either intra-Tenon injection or cellulose sponge whereas the left eye was not (balanced salt solution [BSS] control group) (IACUC protocol ID 13112830).

Filtration Surgery

The rabbits were anesthetized with inhaled isoflurane, and an intramuscular injection of ketoprofen (1.5 mg/kg) was administered for post-procedure pain management. Topical proparacaine 0.5% was applied to each eye. The eyes were then examined for any pre-existing defects, and ophthalmic betadine 5% was then instilled in the eye and on the eyelids.

A limited peritomy (approximately 3 clock hours) was performed with conjunctival dissection in the superotemporal quadrant. For the eyes receiving MMC via injection, an intra-Tenon injection (0.2 mL \times 0.1 mg/mL = 20 μ g total injected, the concentration and dose used in surgical practice for human eyes with axial length below 22.5 mm) was performed superotemporally at the site of the future bleb prior to preparing the rabbit for surgery. The injection into the superotemporal quadrant extended throughout the superior

hemisphere to form a low bleb. The MMC injection site was not manipulated to further spread the MMC. For the eyes receiving MMC application via cellulose sponge, a sponge was soaked in MMC (0.4 mg/mL, standard clinical concentration) and applied to bare sclera in the superotemporal quadrant for 4 minutes. In both groups, the application site was irrigated with BSS (15 mL total irrigation) to remove excess MMC from the ocular surface. The anterior chamber was deepened using Healon (Abbott Medical Optics, Inc., Santa Ana, CA) on a 30-gauge needle.

A 23-gauge hypodermic needle was used to create a scleral tunnel 1 mm posterior to the limbus for insertion of a 22-gauge FEP angiocatheter (BD Insyte; Becton, Dickinson and Company, Franklin Lakes, NJ) into the anterior chamber. The catheter was beveled anteriorly and inserted such that the distal end would cross the pupillary margin to avoid tube-iris capture. The tube was secured to the scleral bed with 10-0 nylon suture and the peritomy closed using the same 10-0 nylon suture (Ethicon, Inc., Cincinnati, OH). Efflux of fluid into the subconjunctival space was confirmed. Successful closure was verified using a Seidel test. In both groups, the left eye of each animal underwent the identical procedure as detailed above except no MMC was utilized. In all eyes, 50 mg ceftazidime (concentration of 200 mg/mL, 0.25 mL total) and 2.5 mg dexamethasone (concentration of 10 mg/mL, 0.25 mL total) was injected into the inferior subconjunctival space at the end of surgery. This was to provide infection prophylaxis and control postoperative inflammation. All animals were sacrificed for histopathology at 4 weeks after surgery.

The intraocular pressures were measured using the iCare TONOVET rebound tonometer (iCare USA, Raleigh, NC) preoperatively at the time of each MMC injection and then every week after for a total of 4 weeks. At the time of intraocular pressure measurement, the blebs were examined for signs of infection or extrusion of the tubes. There were no complications of this kind during the entire experiment. Blebs were formed without leak, infection, or extrusion of the tube in all patients. The rabbits were euthanized at 4 weeks. Ketamine (33 mg/kg) and xylazine (3 mg/kg) were injected intramuscularly to obtain anesthesia, and Euthasol solution (Virbac AH, Inc., Fort Worth, TX) was then injected intravenously. The eyes were then enucleated and sent for pathologic examination.

Histology

All rabbits were euthanized at the end of the 4-week study. All eyes were enucleated and immediately immersed in 10% neutral buffered formalin for at

least 1 week. The eyes were processed and embedded in paraffin blocks by using standard protocols. The angiocatheter was used as the marker for tissue sectioning in order to ensure consistency among analyzed sections in rabbit eyes exposed to MMC (injected or sponge-applied MMC, right eye) and not exposed to MMC (BSS control, left eye). Surgically treated eyes were also compared against a collection of non-surgically treated eyes (non-injured control) of similar age (3–4 months) stored in the laboratory, which have served as control eyes for prior rabbit model experiments within the same laboratory. For the non-injured control eyes, sections allowing detailed evaluation of the angle structures of the eye served as comparables, as the angle structures are relatively preserved in organization and characteristics at all locations within a non-operated eye. Tissue sections (5 μ m) were stained with hematoxylin and eosin (H&E) staining and analyzed for general tissue and cellular morphology and vascularity. The level of fibrosis was measured by collagen deposits evaluated by Masson's trichrome staining (for collagen content) and Picrosirius red staining (for alignment and organization) compared with that of the treatment and/or uninjured tissue by using MetaMorph analysis software (Molecular Devices, LLC, San Jose, CA). Acute inflammation was defined as the presence of neutrophils and chronic inflammation by the presence of plasma and monocytic cells (0, none; 1, slight; 2, moderate; 3, abundant). In both situations, the scale was the relative level of cells per high-power field as previously described.⁵ All slides were analyzed for any evidence of intra- or extraocular abnormalities by a masked examiner.

Collagen Content

Masson's trichrome staining and MetaMorph analysis were used to assess collagen content. Stained histological sections were compared with those of the non-injured control eyes; at all times, the color was maintained to compare the blue- and red-stained areas. The final output was integrated intensity based on total area and staining intensity at individual pixels. All histological sections were stained at the same time to eliminate staining variations.

Collagen Alignment and Organization

Picrosirius red staining was used to assess alignment and organization in intact histological sections. Briefly, picric acid (Sigma-Aldrich, St. Louis, MO) was dissolved in 500 mL of distilled water. To this, 0.1 g of Sirius Red F3BA was added per 100 mL (Sigma-Aldrich). Paraffin-embedded tissue sections

were rehydrated and stained with picric acid. Collagen fibrils were then evaluated by means of polarized light microscopy for both collagen fibril thickness and coherence alignment. Polarization microscopy reveals closely packed thick fibrils of type I collagen fibers as red–orange intense birefringence in the hypertropic tissue, with thin, short, loose fibrils appearing yellow–green. Distribution of fibrils in terms of thickness (cross-sectional area) and arrangement in terms of length were quantitatively analyzed using MetaMorph. Histological sections of non-injured control eyes served to set the threshold against which the surgically treated eyes (both exposed and not exposed to MMC) were measured. Percent staining of mature fibers was determined by comparing the total staining intensity of the birefringence (area of staining summed for intensity of pixel) of histological sections compared with the sections of the non-injured control eyes.

Cellularity and Goblet Cell Count

The degree of subconjunctival cellularity was assessed in all treatment groups. Three randomly selected sections from the conjunctival sample for each rabbit eye were chosen for analysis, and the number of goblet cells per high-power field (40×) were then counted. Averages were obtained for all sample groups and for each individual rabbit.

Neovascularization

Histological assessment of the injured site and morphometric quantification of the number of capillaries were carried out for low-power fields. Immunostaining for CD31 was performed to confirm the neovascularization.

Statistical Analysis

The IOP data collected during the postoperative period prior to sacrificing the rabbits underwent two-tailed, independent *t*-tests to determine statistical significance between the injection group and the sponge group. The quantitative data collected from the tissue samples underwent similar analyses. One-way analysis of variance (ANOVA) was compared on all data sets. Posteriori testing (Tukey's honestly significant difference) was used to confirm where the differences occurred between groups on all groups that showed an overall significant difference. Data are represented as mean and standard error. Overall *P* values were calculated using GraphPad Prism 6 (GraphPad Software, San Diego, CA). Statistically significant

differences were designated by a significance criterion at or below $P < 0.05$.

Results

For the 4-week postoperative period there were no serious ocular complications, such as endophthalmitis. Rabbit 1 in the injected MMC group developed hypotony without a flat anterior chamber. The IOP recordings obtained preoperatively and during the 4-week postoperative period are shown in [Table 1](#). The average postoperative IOP for intra-Tenon injection of MMC was 6.1 mm Hg compared to 9.4 mm Hg for the sponge-applied method, a difference that was statistically significant ($P = 0.04$). In the injected MMC group, rabbits 2 and 3 developed elevated IOP after glaucoma filtration surgery (GFS) compared to their preoperative IOP levels; however, all of the BSS controls developed higher IOP after GFS without MMC. Three rabbits (1, 4, and 5) in the sponge-applied group developed elevated IOP after GFS with MMC compared to their preoperative IOP levels, and three of the BSS controls (1, 2, and 5) also developed elevated IOP after GFS without MMC. The average IOP in the injected MMC group was lower when compared against their BSS controls. The average IOP in the sponge-applied group was slightly higher when compared against their BSS controls.

The injected and sponge-applied MMC treatments were compared against the non-injured control eyes that did not undergo GFS and the control group of eyes that underwent GFS without the use of MMC, instead receiving an injection of BSS ([Table 2](#)). Injected MMC suppressed inflammation and fibrosis, or collagen deposition, more than the sponge-applied method did. The BSS control demonstrated the highest levels of inflammation, fibrosis, and elastic fibers, which would be expected as no MMC was given to suppress the inflammatory and fibrotic response.

The histological analysis of the specimens involved section evaluations utilizing H&E staining ([Fig. 1A](#)). The rabbits receiving an injection of MMC displayed a greater number of goblet cells at the bleb location compared to the sponge-applied MMC eyes ($P < 0.0001$). Of note, the BSS control eyes displayed a lower number of goblet cells compared to the injected MMC group, but the difference was not statistically significant ($P = 0.08$) ([Fig. 1B](#)). The rabbits subjected to injected MMC also showed a decrease in vascularity compared to the non-treated surgical eyes (BSS control) ($P = 0.0039$). Importantly, in both treatment groups, and even the control treatment group,

Table 1. IOP Recordings of Injected MMC and Sponge-Applied MMC Groups With Corresponding Control Eyes from the Respective Groups

Rabbit	BSS Control Groups, IOP Readings (mm Hg)				Treatment Groups, IOP Reading (mm Hg)								
	Pre-Op	Week 1	Week 2	Week 3	Week 4	Average Post-Op	Rabbit	Pre-Op	Week 1	Week 2	Week 3	Week 4	Average Post-Op
Injected group													
1	9	9	10	15	8	10.5	1	7	3	4	3	2	3.0
2	7	7	9	14	9	9.8	2	6	6	10	13	13	10.5
3	6	7	17	15	11	12.5	3	5	2	6	13	5	6.5
4	6	8	8	10	7	8.3	4	8	8	2	5	3	4.5
5	5	5	6	7	6	6.0	5	8	4	5	7	7	5.8
Average						9.4	Average						6.1
Sponge-applied group (control eye)													
1	7	7	8	8	6	7.3	1	6	3	11	10	7	7.8
2	8	7	8	8	11	8.5	2	11	10	11	10	11	10.5
3	14	8	7	13	10	9.5	3	12	5	8	11	9	8.3
4	11	9	8	12	11	10.0	4	4	10	10	12	10	10.5
5	10	10	9	12	12	10.8	5	6	9	8	11	11	9.8
Average						9.2	Average						9.4

Table 2. Comparison of Inflammation, Elastic Fiber Thickness, and Fibrosis Among GFS With MMC, GFS Without MMC, and Non-Surgical Groups

	Non-Injured Control	BSS Control	MMC	
			Sponge Application	Intra-Tenon Injection
Inflammation	-	+++	+	+/-
Elastic fibers (thickness)	+/-	+++	+	++
Fibrosis	-	+++	+	+/-

-, Absent; +/-, weakly present; +, ++, +++, present in increasing amounts (graded by masked observer).

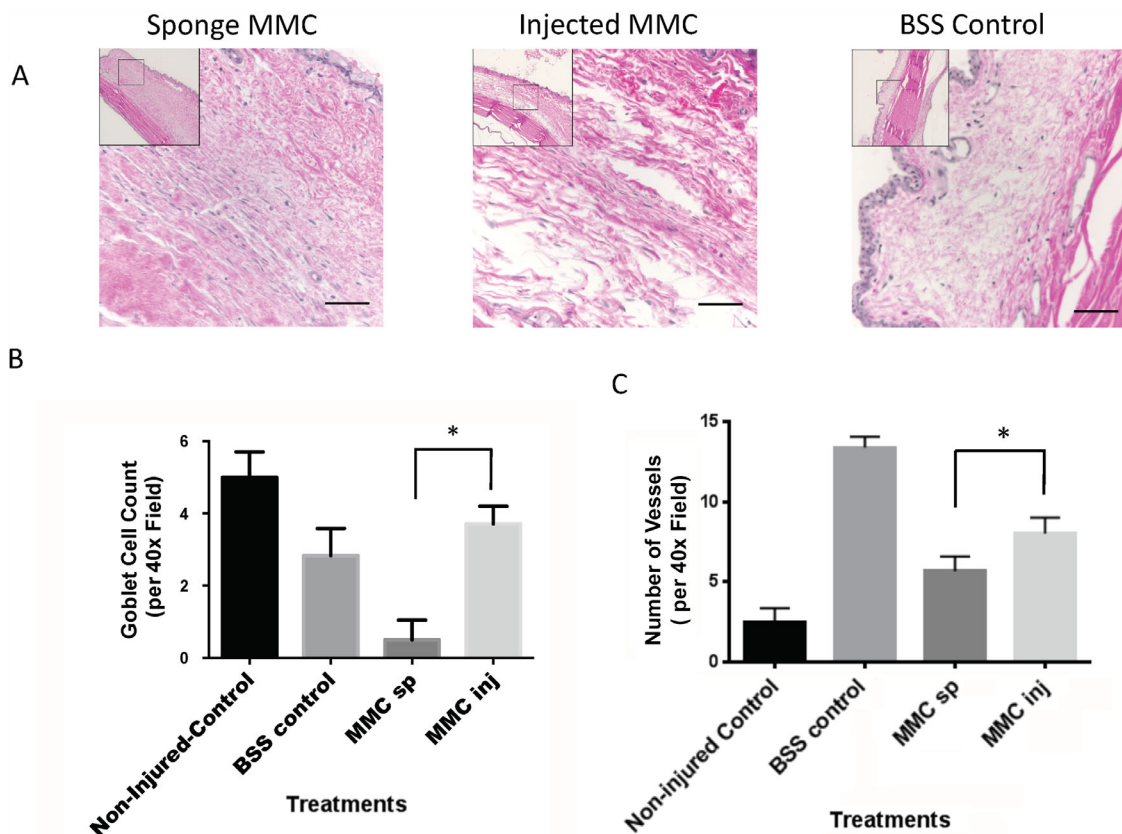


Figure 1. Goblet cell counts of non-surgical control, BSS control, injected MMC, and sponge-applied MMC eyes. (A) H&E staining demonstrated increased cellularity of sponge-applied MMC in the rabbit eye (40× image from 10× image in the upper left corner; images shown are representative of all five treated rabbits). (B) Goblet cell count (per high-power field) showed a statistically significant decrement in all surgeries, but especially for the sponge treatment. The injected treatment showed significantly greater numbers of goblet cells compared to the sponge treatment. ($N = 5$; $\pm SD$; $P < 0.05$ as stated). (C) Vessel presence (per high-power field) demonstrated an angiogenic response after intervention, but this was significantly less in the MMC treated eyes regardless of route of administration. ($N = 5$; $\pm SD$; $P < 0.05$ as stated).

the number of goblet cells was reduced compared to untreated eyes, suggesting a comorbidity inherent in the procedure. Of note, the injected MMC group displayed a greater vascularity compared to the sponge-applied MMC group, but the difference was not statistically significant ($P = 0.394$) (Fig. 1C).

The rabbits subjected to sponge-applied MMC showed an increase in collagen at the bleb location

compared to the injected MMC eyes ($P = 0.01$) (Fig. 2). The Masson’s trichrome stain was used to evaluate collagen content (Fig. 2A), which was highest in the BSS control group versus all other groups. Of note, there was no statistically significant difference when comparing the injected MMC group to the non-injured control group ($P = 0.3828$), but there was a statistically significant difference between the

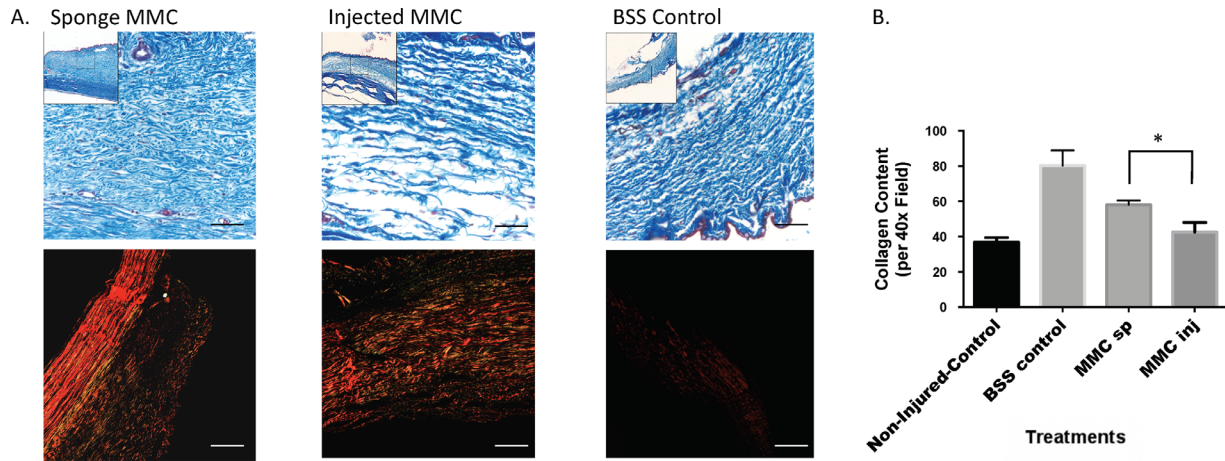


Figure 2. Collagen content and alignment of non-surgical control, BSS control, injected MMC, and sponge-applied MMC eyes. (A) Masson’s trichrome staining (upper panels) revealed the collagen content in rabbit eyes injected with MMC and showed lower amounts of collagen deposition compared to the sponge-applied group. Picrosirius red stain analyses (lower panels) assessed the collagen arrangement and density in rabbit eyes receiving sponge-applied MMC. Note the densely packed and long, thick fibers of type 1 collagen (bright red–orange) in the sponge-applied MMC group versus the loosely packed collagen with thin, short fibrils (yellow–green) in the injected MMC group (40× image from 10× image in the upper left corner; images shown are representative of all five treated rabbits). (B) Quantitation of the collagen content from Masson’s trichrome staining confirm that all surgeries significantly increased the collagen content, although MMC treatment significantly limited the increase, with the injected MMC technique showing a reduction compared to sponge-applied MMC. (N = 5; ±SD; P < 0.05 as stated.)

sponge-applied MMC group versus the non-injured control group ($P < 0.001$) (Fig. 2B). The collagen fibrils were more organized within the sponge-applied MMC group compared to the injected MMC group, which was confirmed by Picrosirius red staining (Fig. 2C). The collagen of the sponge-applied MMC group was tightly packed, with long, thick fibrils of type 1 collagen, in contrast to the injected MMC group, which had loosely packed collagen with thin, short fibrils.

Discussion

The success of GFS depends on a properly functioning bleb. An overfiltering bleb leads to hypotony, increasing the risk for choroidal detachment, maculopathy, and endophthalmitis.⁶ An underfiltering bleb results in a higher, unacceptable intraocular pressure with possible progression. Both scenarios are considered surgical failures. It is evident that bleb morphology, such as size, cellularity, and vascularity, requires a precise interplay of the inflammatory cascade in the postoperative healing phase to create a properly functioning bleb.⁷ Antimetabolite medications, such as MMC, have been used to dampen an exuberant inflammatory response to promote the formation of a filtering bleb with the desired characteristics.² This study compared a newer approach

to administering MMC via an intra-Tenon injection against the standard-of-care approach of placing a cellulose sponge soaked in MMC onto a bare scleral bed for a specific amount of time, as in the New Zealand White Rabbit model. Note that 0.4 mg/mL MMC was applied by sponge versus 0.1 mg/mL MMC by injection to account for cell toxicity thresholds. It has been shown that MMC injections of 0.2 mg/mL were sufficient to induce severe morphological alterations in subconjunctiva cells,⁸ whereas levels as high as 0.5 mg/mL of sponge-applied MMC did not induce signs of cellular toxicity.⁹

An injection of MMC resulted in a statistically significant lower IOP than did sponge-applied MMC. A potential explanation for this finding is that injection results in a more diffuse bleb compared to the pocket that is formed from dissecting through conjunctiva and the Tenon to the sclera. It has been reported that good intraocular pressure control after filtration surgery is typically associated with low-lying diffuse blebs.¹⁰ During this experiment, no formal grading or photography of the blebs was performed. This remains an avenue for future investigation. Also, MMC is known to affect the ciliary body; therefore, this finding is potentially related to increased effects on the ciliary body resulting in hyposecretion of aqueous humor or increased uveoscleral outflow. Interestingly, the injected MMC group demonstrated a lower average postoperative IOP compared to the BSS

control; however, there was no appreciable change in the average IOP in the sponge-applied MMC group versus the BSS controls. This finding further underlies the premise of this study that the amount of MMC delivered through the sponge-applied method is unpredictable and may ultimately affect the overall success of GFS.

Goblet cell counts were greater in the injected MMC group compared to the sponge-applied MMC group. Both MMC groups demonstrated lower goblet cell counts compared to the non-injured control group. The underlying cause for the difference in the MMC groups could be due to the concentrated, focal application in the sponge-applied MMC group versus the broader and less concentrated application of MMC in the injected group. This is important, as ocular surface disease (OSD) is a significant source of the morbidity in patients following glaucoma filtration surgery. Goblet cells play a crucial role in tear film stability and in the maintenance of a healthy ocular surface. Patients with low goblet cell counts have been reported to have reduced tear breakup times.¹¹ Interestingly, the BSS control eyes demonstrated a lower number of goblet cells compared to the injected MMC group. Although this result was not statistically significant, it highlights the interplay of goblet cells and medication-induced toxicity. Additionally, inflammation has been associated with a reduction in the goblet cell population, which has been demonstrated through impression cytology in pathological inflammatory states such as keratoconjunctivitis sicca, ocular cicatricial pemphoid, and Stevens–Johnson syndrome.¹² We hypothesize that the higher number of goblet cells in the injected MMC group compared to the sponge-applied MMC and BSS control groups represents a relative suppression of the inflammatory response, but local toxic effects such as the goblet cell counts were still lower than in the non-injured control group. Common presenting symptoms following trabeculectomy with MMC are a sensation of dry eye, a foreign body, or grittiness, as well as redness. Both mean tear breakup time (5.32 seconds) and Schirmer testing (6.14 mm/5 minutes) have been found to be below normal in such patients.¹³ Therefore, a thorough evaluation of the ocular surface is crucial in the preoperative period. It should also be noted that topical IOP-lowering medications, which can contain the preservative benzalkonium chloride, contribute to OSD. A patient with pre-existing OSD may be a better candidate for injected versus sponge-applied MMC given these findings.

Collagen content was appreciably lower in the injected MMC group versus the sponge-applied MMC group. This finding is likely related to the increased exposure time and area, as the MMC disperses after it

is injected whereas the sponge is applied to a focal area. This also may correlate with a lower IOP in the injected MMC group, as injection is thought to produce a more diffuse bleb. The size of a bleb has been shown to directly correlate with the treatment area of MMC.¹⁴ As expected, our BSS control group demonstrated the highest collagen content due to the lack of fibroblast suppression compared to the MMC groups. Interestingly, the injected MMC group did not show a statistically significant difference in collagen content when compared to the non-injured control group, whereas the sponge-applied MMC group did show a higher collagen content than the non-injured control eyes which was statistically significant. This finding demonstrates that the injected MMC suppressed the fibroblastic response closer to the level of a non-surgical eye, which is key to the success of GFS.

The decrease in bleb vascularity was consistent with prior experience in the literature. The use of MMC during GFS results in the creation of avascular blebs. Recent studies have specifically targeted angiogenesis to decrease vessel formation and capillary permeability, thus reducing the systemic delivery of inflammatory mediators. MMC has been demonstrated to outperform bevacizumab with regard to IOP control; however, there was no significant difference in bleb vascularity and morphology.¹⁵ Although MMC has enhanced the success of trabeculectomy surgery, the avascular blebs have been prone to transconjunctival oozing and delayed leaks that can lead to endophthalmitis.^{2,3}

One of the main omissions of this study was not documenting or photographing the appearance of the bleb in the postoperative period. Common classification schemes for assessing bleb morphology are the Indiana Bleb Appearance Grading Scale and the Moorfields Bleb Grading System. In future studies, one of these classification schemes could be utilized during the postoperative period to assess the hypothesis that injected MMC produces a more diffuse bleb compared to the sponge-applied method. Another limitation is the overall sample size. A larger sample size would allow for a more robust clinical and histological evaluation. However, this sample size has been previously used in the literature, and it is believed that the results relating to goblet cell and collagen content would hold true for a larger sample size.

This study shows that intra-Tenon injection can be an alternative method of administering MMC. An injection of MMC led to a greater reduction in intraocular pressure and inhibition of fibroblasts, thus reducing the collagen content and fibrosis when compared to the standard method of application via a cellulose sponge onto bare sclera. The associated

reduction in goblet cell populations, as observed for both the injected and sponge-applied MMC methods, could lead to increased morbidity related to MMC toxicity, such as dry eyes, reduced visual acuity, corneal decompensation, and patient discomfort. However, patients with OSD may benefit from the injected MMC method, as a greater number of goblet cells were preserved in our study. The increase in bleb vascularity in the intra-Tenon injection group versus the sponge-applied group increases the risk of bleb failure in the former. Further studies are needed to standardize the concentration of injected MMC to minimize these effects while still providing the desired anti-inflammatory and antifibrotic action to create a properly functioning bleb.

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