Topical Silver Nanoparticles Result in Improved Bleb Function by Increasing Filtration and Reducing Fibrosis in a Rabbit Model of Filtration Surgery

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Submitted: March 16, 2013

Accepted: June 6, 2013

Citation: Butler MR, Prospero Ponce CM, Weinstock YE, Orengo-Nania S, Chevez-Barrios P, Frankfort BJ. Topical silver nanoparticles result in improved bleb function by increasing filtration and reducing fibrosis in a rabbit model of filtration surgery. *Invest Ophthalmol Vis Sci*. 2013;54:4982–4990. DOI:10.1167/iovs.13-12047

PURPOSE. To compare the effects of silver nanoparticles (AgNPs) and mitomycin C (MMC) on intraocular pressure (IOP) and external, histologic, and immunohistochemical bleb characteristics in a rabbit model of filtration surgery.

METHODS. Filtration surgery with concurrent topical application of either AgNPs or MMC was performed on 14 pigmented Dutch Belted rabbits. IOP and bleb characteristics were compared on postoperative day 1 and at weeks 1 through 6. Hematoxylin and eosin staining and smooth muscle actin (SMA) immunohistochemistry were performed at postoperative week 6.

RESULTS. Average IOP across all time points was reduced 5.8 and 3.8 mm Hg in AgNP- and MMC-treated eyes, respectively. At week 6, IOP was reduced 4.1 and 0.2 mm Hg in AgNP- and MMC-treated eyes, respectively. Blebs were smaller, thicker, and less ischemic in AgNP-treated eyes. AgNP-treated eyes showed less fibrosis and more stromal edema, suggesting increased filtration, and also had fewer SMA-positive myofibroblasts, suggesting reduced bleb contraction. AgNP-treated eyes showed more lymphocytes than MMC-treated eyes. There were few complications in both groups.

CONCLUSIONS. In a rabbit model of filtration surgery, AgNPs are a reasonable alternative to MMC as adjunctive therapy. Compared to MMC, AgNPs result in an improved and sustained reduction of IOP and promote blebs with decreased fibrosis and ischemia as well as increased filtration despite a smaller overall size. This combination may offer an opportunity to promote long-term surgical IOP reduction with an improved complication profile.

Keywords: filtration surgery, glaucoma, silver nanoparticle, mitomycin C, rabbit

Glaucoma is the second most common cause of blindness worldwide and is expected to affect 80 million individuals by 2020.¹ The only currently modifiable risk factor for glaucoma is intraocular pressure (IOP), and reduced IOP is associated with delays in both glaucoma onset and progression.^{2–4} When medical therapy fails, surgical IOP lowering is often required. The surgery most commonly performed to lower IOP is trabeculectomy, which is a form of filtration surgery and involves creating a fistula to divert aqueous humor from the anterior chamber to the subconjunctival space. The structure that forms, called a “bleb,” becomes the new site of aqueous filtration. Fibroblastic proliferation, subconjunctival fibrosis, scarring of the trabeculectomy flap, and remodeling of collagen with contraction of tissue in and around the bleb are the major causes of surgical failure.^{5,6} To reduce scar tissue formation, topical antifibrotics such as 5-fluorouracil and mitomycin C (MMC) are used at the time of trabeculectomy surgery.^{7–9} MMC is the most effective agent, and the improved outcomes associated with its use appear to be mediated by reduced fibroblast proliferation and bleb fibrosis.^{10–13}

Unfortunately, an increased incidence of potentially devastating complications such as bleb leak, endophthalmitis, and

hypotony has been attributed to the use of MMC.^{8,14,15} Therefore, there is significant need for alternative agents that provide increased surgical success without a concurrent increase in serious complications. Many have been tried in both animals and humans with variable degrees of success.^{16–31}

Elemental silver has long been used as a medical agent for its anti-infective properties, and has been used in ophthalmology in silver nitrate form as an agent to prevent ophthalmia neonatorum. More recently, silver nanoparticles (AgNPs, diameter < 100 nm) that retain broad spectrum antibacterial activity have been developed.^{32,33} While the exact mechanism of action of AgNPs is not entirely clear, they appear to interact with multiple bacterial cellular components including the cell wall, DNA, and ribosomes.^{34,35} In addition to antibacterial properties, AgNPs possess other properties with potential medical benefits. For example, AgNPs have been used as an adjunctive agent after burn injuries, and they lead to accelerated wound healing with reduced inflammation.³⁶ Other evidence suggests that the anti-inflammatory effects of AgNPs allow for wound healing with diminished scar tissue formation and lead to inhibitory effects on both fibroblast proliferation and cytokine production.^{37–39} Multiple studies suggest that

AgNPs are relatively safe, but large particle diameters and high concentrations have been associated with increased cyto- and genotoxicity both in vivo and in vitro.^{40–42} Finally, studies of topical ocular exposures in mice and rabbits suggest that AgNPs are well tolerated.^{43,44}

The potential combination of decreased scarring, reduced inflammation, fibroblast inhibition, infection prophylaxis, and high tolerability suggests that AgNPs would be beneficial during trabeculectomy surgery. In particular, AgNPs may allow healing to occur without aggressive scar tissue formation or subconjunctival fibrosis, thereby promoting bleb survival. To test this hypothesis, we compared the effects of AgNPs and MMC in an established rabbit model of filtration surgery.⁴⁵ We report that AgNP-treated rabbits had lower IOP, as well as thicker, smaller, and less ischemic blebs. Histologically, AgNP-treated rabbits had reduced fibrosis and myofibroblast proliferation. AgNP-treated animals also had increased lymphocytic infiltrate and increased edematous intrastromal fluid-filled spaces within the surgical bleb, likely representing increased filtration. AgNPs show potential as a novel adjuvant during filtration surgery.

METHODS

Animal Husbandry and Surgical Procedure

All animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and standards set forth by the Baylor College of Medicine Institutional Animal Care and Use Committee. Protocols were approved by the Baylor College of Medicine Institutional Animal Care and Use Committee. Fourteen pigmented Dutch Belted rabbits weighing between 2 and 3 kg underwent filtration surgery in the right eye performed by a single surgeon (BJF) according to an established protocol with minor modifications during a 1-year period from August 2011 to August 2012.⁴⁵ Topical anesthetic (proparacaine 0.5%) was applied to the right eye of awake animals and the IOP recorded with a Tonopen (Reichert Technologies, Depew, NY). At least two measurements were taken, and the average of all recordings was documented as the IOP. Because significant rotation of the globe is common in anesthetized rabbits, the 12 o'clock position of the limbus was identified with a marking pen prior to anesthesia. Animals were then anesthetized with a weight-based intramuscular injection of ketamine 45.2 mg/mL, xylazine 9.2 mg/mL, and acepromazine 0.77 mg/mL. The right eye of the anesthetized animal was then prepped with betadine and a lid speculum placed to expose the entire cornea. A superior 6-0 silk corneal traction suture was placed at the marked area to allow for infraduction of the globe. A limbal peritomy extending approximately 5 mm in each direction from 12 o'clock was created. Posterior dissection was carried out as far as possible to ensure that at least 10 mm of sclera was exposed. The protocol differed from a previously established protocol by reducing the amount of the posterior dissection from 15 mm to at least 10 mm and by omitting the step in which a protective plastic shield is placed over bare sclera and cornea prior to adjuvant placement.⁴⁵ Animals were randomized 1:1 to one of two treatment groups in which the adjuvant was applied with a large soaked Weck-cel sponge (dry size 8 × 8 mm; Beaver-Visitec International, Inc., Waltham, MA) directly on bare sclera in the subconjunctival space:

1. 4-minute application of MMC, 0.4 mg/mL, which was then irrigated with saline;
2. 4-minute application of AgNPs, 10 nm average diameter, 0.1 mg/mL colloidal solution in water (Plasmachem, Berlin, Germany), which was not irrigated.

The surgeon was aware of the adjuvant because of the difference in color of liquid MMC (clear) and AgNPs (ochre). A 22-gauge intravenous catheter was then inserted into the anterior chamber through a 3 mm scleral tunnel to ensure patency of the sclerostomy. The posterior edge of the catheter was trimmed and fixed to the sclera with a single 10-0 nylon suture. The peritomy was closed with three 10-0 nylon sutures (one central mattress at 12 o'clock and one suture at each limbal wound edge). Rabbits then received a weight-based intramuscular injection of buprenorphine 0.3 mg/mL and were allowed to recover on a warming blanket.

Rabbits were evaluated on postoperative day 1 (POD1) and at postoperative weeks 1 through 6 (POW1–POW6) by two investigators (MRB and BJF). At all time points, topical anesthetic was placed in the operative eye, and recordings were made of IOP with a Tonopen (Reichert Technologies). We recorded the following bleb characteristics: thickness (the thickness of the surface of the bleb tissue; 3-point scale where 0 = thin, 1 = moderate, and 2 = thick); height (vertical extent of the bleb from the limbus to the superior bleb edge in millimeters); width (horizontal extent of the bleb along the limbus in millimeters); diffuseness (measure of how much of the treated area the bleb involved); vascularity of the bleb, bleb edge, and surrounding nonbleb tissue (all evaluated on a 3-point scale where 0 = minimal, 1 = moderate, and 2 = prominent); anterior chamber depth (0 = flat, 1 = shallow, 2 = deep); and complications.⁴⁶ For each characteristic, the same two investigators made separate and independent measurements, and the average was recorded. Since the surgeon was involved the measurements (BJF), one reading was not masked. Animals were assessed for pain and distress at all time points. After the POW6 examination, all animals were euthanized with a lethal dose of beuthanasia D administered to the ear vein. Operative eyes were promptly enucleated and placed in 10% formalin.

Histologic and Immunohistochemical Analysis

Enucleated eyes were labeled by the pathologist in a masked fashion so that the treatment group could not be determined from the slide label. Fourteen eyes were grossly examined to include sections of the bleb and adjacent tissues for histologic examination. The selected areas of the eye were then processed for routine paraffin-embedded tissue. Three levels of each block were obtained and stained with standard hematoxylin-eosin (H&E) stain. Unstained slides in between levels were then used for immunohistochemistry. Slides were deparaffinized, rinsed with deionized water, and placed in Dako Wash Buffer (Carpinteria, CA). Antigen retrieval was performed using Dako Target Retrieval Solution. Endogenous peroxidase activity was blocked with 3% H₂O₂, and endogenous biotin was blocked using Vector Avidin/Biotin Blocking Kit (Burlingame, CA). Nonspecific protein binding was blocked with Dako Serum Free Protein Block, and the slides were incubated with mouse ready-to-use antismooth muscle actin, clone 1A4 (Biogenex, Fremont, CA) for 30 minutes at room temperature. Slides were washed and incubated for 30 minutes with Vector biotinylated anti-mouse IgG diluted 1:150 in Dako Antibody Diluent. Slides were then washed and incubated for 30 minutes with Vector VECTASTAIN Elite Standard ABC Kit prepared in Dako Wash Buffer according to manufacturer's directions. Slides were washed in wash buffer and incubated for 5 minutes with Dako 3,3'-Diaminobenzidine (DAB)+ Substrate Chromogen System prepared according to manufacturer's directions. Slides were rinsed in deionized water, placed in wash buffer, counterstained with Hematoxylin 2 (Richard-Allan Scientific, Kalamazoo, MI), dehydrated, and coverslipped.

Fibrosis (defined as the presence of collagen deposition within the tissue) and edema (defined as fluid-filled spaces

FIGURE 1. Intraocular pressure (IOP) after filtration surgery is lower in eyes treated with AgNPs. IOP (mm Hg) was measured with a Tonopen preoperatively (POD 0) and at each postoperative examination (POD 1, POW 1–POW 6; see Methods). MMC- and AgNP-treated eyes are presented in *black* and *gray*, respectively; $n = 7$ for both groups. Preoperative mean IOPs were similar between the two groups. After filtration surgery, IOP was reduced when treated with either AgNPs or MMC at multiple time points (*asterisks*, paired *t*-test compared to baseline IOP, $P < 0.05$). Overall, IOP reduction in AgNP-treated eyes was greater than in MMC-treated eyes (repeated measures ANOVA, $P = 0.02$). At later time points (POW 5, POW 6), the IOP of MMC-treated eyes increased, suggesting late surgical failure of MMC-treated eyes. Error bars represent one SEM.

within the tissue stroma that appears as cleared stroma when seen histologically) were evaluated through the middle of the bleb over the sclera in all eyes to allow for direct comparison of tissue. Fibrosis and edema were graded as 0 when absent, 1+ when involving less than 50% of tissue, 2+ when involving between 50% and 80% of tissue, and 3+ when involving $\geq 80\%$ of the tissue. Fibroblasts were easily identified by H&E staining and were scored according to the same scale. Lymphocytes, eosinophils, and vessels, which were less abundant, were graded 0 when absent, 1+ when present in 0 cells to 1 cell per high-power field in 5 fields, 2+ when present in 2 to 4 cells per high-power field in 5 fields, and 3+ when present in >5 cells per high-power field in 5 fields. For these measurements a high-power field was defined as a single area at $\times 40$ magnification. Myofibroblasts were identified by immunostaining with SMA and graded according to the same scale as lymphocytes, eosinophils, and vessels.

External Photography

Photographs were obtained with a Nikon D3 camera (Nikon, Tokyo, Japan) with an AF Micro-NIKKOR 60 mm f/2.8D lens

(Nikon) at focal distances between 0.25 and 0.50 m. Bright ambient lighting without a flash was used for all photos. Aperture, shutter speed, and ISO were adjusted for depth of field and exposure optimization.

Statistical Analysis

Statistical analysis was performed using parametric and nonparametric statistics in SPSS version 20 (IBM, Armonk, NY). To measure the effect of surgery on IOP, a paired *t*-test with a cutoff of $P < 0.05$ was used. For analysis of continuous data of repeated measures, ANOVA with a cutoff of $P < 0.05$ was used. For analysis of ordinal data, nonparametric statistics at multiple time points (Mann-Whitney test) with a cutoff of $P < 0.05$ were used.

RESULTS

AgNP-Treated Eyes Have a Lower IOP Than MMC-Treated Eyes

Since IOP is a critical risk factor for glaucoma development and progression, we first sought to compare the reduction in IOP

TABLE 1. Intraocular Pressure After Filtration Surgery in MMC- and AgNP-Treated Eyes

	POD 0		POD 1		POW 1		POW 2		POW 3		POW 4		POW 5		POW 6		
IOP	MMC	AgNP	MMC	AgNP	MMC	AgNP	MMC	AgNP	MMC	AgNP	MMC	AgNP	MMC	AgNP	MMC	AgNP	rmANOVA
Mean	18.7	18.7	13.1	12.3	15.1	13.3	14.1	11.0	13.2	12.6	13.2	12.6	17.1	14.1	18.5	14.6	0.020
Min	16.5	14	9	10	8.5	11	7	10	5.5	8.5	8.5	11	14	10.5	11	12	
Max	21.5	22.5	18	15.5	26.5	18.5	20.5	13	22.5	18.5	15.5	15.5	19.5	19.5	36	20	
Std. dev.	1.6	3.9	2.7	2.2	5.8	2.7	4.7	1.0	5.7	3.2	1.8	1.7	2.3	3.6	8.2	3.4	

Mean, minimum (Min), and maximum (Max) IOP (mm Hg) with standard deviation (Std. dev.) for both MMC- and AgNP-treated eyes at all time points after surgery (POD 1 and POW 1–6). POD 0 represents the day of surgery; $n = 7$ for both groups. Both MMC- and AgNP-treated eyes had a statistically significant reduction in IOP at multiple time points, suggestive of successful filtration surgery, but the reduction in AgNP-treated eyes was greater (repeated measures ANOVA [rmANOVA], $P = 0.020$; see Fig. 1). IOP was lower in AgNP-treated eyes than MMC-treated eyes at every time point. IOP levels were maintained in AgNP-treated eyes but tended to rise at later time points in MMC-treated eyes. By week 6, the average reduction of IOP in MMC-treated eyes had decreased to 0.2 mm Hg, suggesting bleb failure.

TABLE 2. Postoperative Bleb Characteristics

	POD 1		POW 1		POW 2		POW 3		POW 4		POW 5		POW 6	
	MMC	AgNP	MMC	AgNP	MMC	AgNP	MMC	AgNP	MMC	AgNP	MMC	AgNP	MMC	AgNP
Bleb height, mm	5.3	4.9	5.3	4.0	4.7	3.4	3.4	2.4	3.0	2.0	2.1	2.1	1.7	1.3
Bleb width, mm	10.0	9.0	9.3	6.7	9.0	4.9	6.6	3.6	6.1	2.7	4.0	3.0	2.9	1.9
Bleb diffuseness, %	100	96	86	76	81	49	74	30	53	20	41	18	16	11
Bleb thickness, 0–2	1.2	0.9	0.6	0.9	0.3	0.9	0.1	1.0	0.2	1.0	0.2	0.9	0.3	0.9
Vascular nonbleb, 0–2	1.7	1.2	1.4	1.1	1.5	0.9	1.4	0.9	1.4	1.0	1.3	0.9	1.2	0.9
Vascular bleb edge, 0–2	1.2	0.7	1.2	1.0	1.4	0.9	1.2	0.7	1.1	0.9	1.3	0.9	1.1	0.9
Vascular bleb, 0–2	0.7	0.5	0.4	0.7	0.1	0.9	0.1	0.7	0.2	0.6	0.4	0.8	0.3	0.8

Mean results are reported for each characteristic. Bleb height was similar between the two groups, but bleb width was significantly greater in the MMC-treated group (repeated measures ANOVA, $P = 0.035$), with smaller blebs in the AgNP-treated group. Blebs were also less diffuse in the AgNP-treated group (ANOVA, $P = 0.049$), and both groups showed a gradual reduction with time. AgNP-treated blebs were of stable, intermediate thickness at all time points while MMC-treated blebs became thin by POW 2 (Mann-Whitney test, $P < 0.05$, for each time point from POW 2 through POW 6). At all time points, AgNP-treated eyes were less vascular than MMC-treated eyes at the bleb edge and at nonbleb tissue. At all time points subsequent to POD 1, the bleb tissue was more vascular in the AgNP-treated group, suggesting fewer ischemic changes. Anterior chamber depth was deep for all eyes at all time points.

achieved with AgNPs to that obtained with MMC. IOP after filtration surgery was significantly lower than baseline at multiple time points in both groups (Fig. 1, Table 1), suggestive of successful surgery. Interestingly, the IOP reduction seen in the AgNP-treated group was greater than that in the MMC-treated group (repeated measures ANOVA, $P = 0.02$), and the average reduction of IOP across all time points was 5.8 mm Hg in AgNP-treated eyes and 3.8 mm Hg in MMC-treated eyes. In both groups, there was a trend toward higher IOPs beginning at week 5. However, whereas the IOP of MMC-treated eyes returned essentially to baseline by week 6 (average IOP reduction = 0.2 mm Hg), the IOP of AgNP-treated eyes

remained reduced (average IOP reduction = 4.1 mm Hg). This trend in IOP elevation in MMC-treated eyes is suggestive of gradual bleb failure.

AgNP-Treated Eyes Have Smaller, Less Ischemic Blebs Than MMC-Treated Eyes

In order to compare the morphologic appearance of the blebs of both groups, we recorded multiple metrics of the bleb appearance at each time point (see Methods section). Overall, bleb characteristics were quite different between the two groups. In general, AgNP-treated eyes had blebs that were

FIGURE 2. External appearance of AgNP- and MMC-treated eyes. Eyes treated with AgNPs (A–C) and MMC (D–F) were photographed at POW 1 (A, D), POW 2 (B, E), and POW 4 (C, F). The animals shown are representative of the time course of healing for both groups. At all time points, AgNP blebs had coloring and vascularity similar to those of surrounding tissues. With time, these blebs gradually became more focal (less diffuse) in appearance (*outline*). MMC blebs (*outline*) were larger and increasingly ischemic (translucent appearance at POW 4, *asterisk*). Note the position of the intravenous catheter in the superior aspect of the anterior chamber in all parts of the figure.

FIGURE 3. Histologic summary of AgNP- and MMC-treated eyes. A scoring system was used to evaluate the bleb areas of seven MMC-treated and seven AgNP-treated eyes. Collagen deposition (Fibrosis), interstitial edema (Edema), total fibroblasts (Fibroblasts), total lymphocytes (Lymphocytes), total eosinophils (Eosinophils), blood vessels (Vessels), smooth muscle actin-positive vessels (Actin_Vessels), and smooth muscle actin-positive myofibroblasts (Actin_Myofibros) were evaluated (see Methods). (A) Mean values with standard deviation. (B) Median values. (A, B) Black bars represent MMC-treated eyes, and gray bars represent AgNP-treated eyes. Reduced fibrosis and increased lymphocyte proliferation were seen in AgNP-treated eyes compared to MMC-treated eyes (Mann-Whitney test, $P = 0.025$, 0.045 , respectively). There were trends toward increased interstitial edema and decreased actin myofibroblasts in AgNP-treated eyes.

smaller, thicker, more vascular, and more focal, whereas MMC-treated eyes had blebs that were larger, more diffuse, and more ischemic. The area surrounding the bleb was more vascular in the MMC-treated group. Both groups showed a gradual reduction in bleb size over time (Table 2). In general, AgNP-treated tissue appeared healthier than MMC-treated tissue and did not have areas of thin, ischemic, translucent tissue (Fig. 2).

AgNP- and MMC-Treated Eyes Have Few Complications

Surgical complications in both groups were low. Three animals treated with MMC had a mild chemical conjunctivitis that resolved spontaneously in two animals. In the third, a mild blebitis without endophthalmitis developed that resolved with topical antibiotic therapy. One AgNP-treated animal had a tube dislocation in which the tube partially migrated into the anterior chamber. There were no instances of shallowing or flattening of the anterior chamber.

AgNP-Treated Eyes Have Less Scarring and More Filtration Than MMC-Treated Eyes

Histologic analysis of enucleated eyes revealed multiple differences between AgNP- and MMC-treated eyes (Fig. 3). Most strikingly, the subconjunctival space of AgNP-treated eyes had less fibrosis (less organized collagen deposition) and more stromal edema or intrableb fluid-filled spaces than MMC-

treated eyes. This edema was uniform throughout the bleb, whereas MMC-treated eyes showed abrupt fibrotic borders with large cystic spaces and contractile myofibroblasts in the wall (Figs. 4A–D). The presence of uniform stromal edema is suggestive of increased filtration throughout the bleb with transconjunctival diffusion of filtered aqueous. Thus, despite a smaller overall appearance, it is possible that AgNP-treated eyes had improved overall filtration, which may explain how the smaller blebs seen in AgNP-treated eyes could also produce a lower IOP. Interestingly, as there was no difference between the two groups with regard to the amount of total fibroblasts, this difference in healing is unlikely to result from a simple reduction of fibroblasts and is more likely due to a change in fibroblast properties, terminal differentiation, or the surrounding tissue environment. To further assess this, we quantified the number of fibroblasts that had transformed into myofibroblasts through immunostaining with smooth muscle actin (SMA). We detected smooth muscle–positive myofibroblasts primarily at the bleb edges in MMC-treated eyes, often surrounding large fluid-filled spaces, which is suggestive of encysted and contractile blebs. In AgNP-treated eyes, there was a reduced number of smooth muscle–positive myofibroblasts, which suggests reduced or altered fibroblast transformation (Figs. 4E, 4F). Since myofibroblasts have contractile properties, this reduced number of myofibroblasts most likely reduced both contraction of the bleb edges and remodeling of the bleb wall. This altered healing environment is also suggested by the finding that AgNP-treated eyes had slightly more lymphocytes than MMC-treated eyes, which were more likely to show an eosinophilic infiltrate (Figs. 4G, 4H).

FIGURE 4. Histologic and immunohistochemical findings for AgNP- and MMC-treated eyes. AgNP- and MMC-treated eyes are presented in the *left* and *right columns*, respectively. (A–D, G, H) Hematoxylin and eosin staining at $\times 10$ magnification (A, B) and $\times 40$ magnification (C, D, G, H). (E, F) Immunohistochemistry using smooth muscle actin (SMA) antibody and DAB chromogen at $\times 20$ magnification. Scale bars are indicated for each part of the figure. The bleb area over the sclera (asterisk) in a typical AgNP-treated eye (A) showing uniform edema and no cyst formation, and in a typical MMC-treated eye (B) showing packed collagen and a cystic bleb. A representative AgNP-treated eye (C) showing moderately edematous stroma and a representative MMC-treated eye (D) showing a densely packed, collagenous, cellular background without edematous stroma. In a

typical AgNP-treated eye (E), SMA is present in smooth muscle in open vessels surrounding the edematous bleb, but no myofibroblastic proliferation is seen. In a typical MMC-treated eye (F), SMA-positive myofibroblasts are seen in the fibrous stroma at the edge of the bleb, suggesting an encysted bleb. Inflammation associated with the edges of the bleb area in a representative AgNP-treated eye (G) showed predominance of lymphocytes with a rare eosinophil. In a representative MMC-treated eye (H), less lymphocytic infiltrate and more eosinophils were seen.

DISCUSSION

Treatment With AgNPs Results in Sustained IOP Reduction

Any improved surgical alternative to MMC should result in at least similar IOP lowering following filtration surgery with reduced complications. In our model of rabbit filtration surgery, MMC-treated animals had IOP that was lower than baseline at all time points, although IOP was essentially the same as baseline by postoperative week 6. Strikingly, AgNP-treated animals also had IOP lower than baseline at all time points. Furthermore, when compared to MMC-treated animals, AgNP-treated animals had a statistically lower IOP. Thus, IOP lowering with AgNPs was superior overall (Fig. 1, Table 1).

We report an increase in IOP at the later time points among MMC-treated eyes, which implies surgical failure. Another extensive study also reported a relatively high failure rate of MMC-treated blebs in rabbits at 1 month after surgery, which is consistent with our findings.⁴⁵ Thus, the maintenance of low IOP at 6 weeks in most AgNP-treated eyes is promising, and has the potential for long-term IOP reduction.

AgNPs Promote Healthy Blebs With Prolonged Survival

AgNP-treated blebs were thicker, less ischemic, and less hypervascular at their borders than MMC-treated blebs (Fig. 2, Table 2). Histologically, 6 weeks after surgery, AgNP-treated eyes also had markedly less fibrosis and collagen deposition than MMC-treated eyes, almost no myofibroblastic transformation, accounting for minimal bleb contraction, and increased interstitial edema suggestive of improved filtration. This combination of findings is consistent with the possibility that AgNPs promote healthier, better-functioning blebs with decreased contractile scar tissue formation. In this context, improved filtration may occur as well, resulting in decreased IOP. In a mouse model of wound healing after thermal injury, wounds treated with topical AgNPs closely resembled normal skin and had reduced hypertrophic scarring in a dose-dependent manner.³⁷ Therefore, it is possible that a more "normal" healing process is occurring in our filtration surgery model as well. Since we did not titrate the dose of AgNPs and since increased AgNP concentration is associated with increasing levels of both effect and toxicity, it is possible that different concentrations of AgNPs would produce different results.^{40–42} Interestingly, we found that AgNP-treated blebs were smaller than MMC-treated blebs, yet had improved survival and lower IOP. This suggests that the quality of filtration may not correlate simply with bleb size, and that smaller but more efficient blebs may be possible with AgNPs. Such blebs are potentially less likely to cause symptoms of bleb dysesthesia and ocular discomfort. Lastly, the blebs of AgNP-treated eyes were thicker, more vascular, and less ischemic than those in MMC-treated eyes. This distinction is critical, as thin and ischemic blebs are thought to represent a significant risk factor for some of the devastating complications of glaucoma surgery, such as bleb leak, hypotony, and endophthalmitis. Thus, AgNP treatment may also result in fewer long-term complications following filtration surgery.

AgNPs Promote a Different Postoperative Histologic Environment Than MMC

Histologic evaluation of AgNP- and MMC-treated eyes showed similar numbers of total fibroblasts. Previous work has implicated both AgNPs and MMC as inhibitors of fibroblast proliferation.^{10,12,38} However, we observed very different patterns of fibrosis (dense collagen deposition in MMC-treated eyes and loose deposition with interstitial edema in AgNP-treated eyes) despite similar numbers of fibroblasts. One possible explanation is that while both AgNPs and MMC reduce fibroblast proliferation, AgNPs have additional effects on the cytokine microenvironment that result in decreased relative fibrosis. Indeed, AgNPs have been implicated as inhibitors of both IL-6 and TGF- β 1, which are potent proinflammatory cytokines.³⁷ Similarly, AgNPs decrease peritoneal adhesions in a surgical model in mice.³⁹ It is possible that an analogous process occurs after filtration surgery, with less fibrosis and fewer fibrotic adhesions. Nevertheless, the relative abundance of lymphocytes in AgNP-treated eyes suggests that there is still some degree of chronic inflammation present. We also observed reduced myofibroblast transformation in AgNP-treated eyes, which contrasts with a previous study suggesting that AgNPs promote myofibroblast transformation.³⁸ That study was conducted on skin tissue, which has more cell types and complexity than conjunctiva. Furthermore, the authors of that report were looking at healing in sites such as skin, which require wound remodeling through myofibroblastic transformation. Thus, the differences in tissue makeup and endpoint objectives may explain the differences between the two studies, and could be further explained by the inflammatory microenvironment, the anatomic difference, or the lubrication of the tissue. Further studies to differentiate the timing and quality of the cytokine environment during the postoperative period may also explain these discrepancies. Since AgNP-treated eyes had smaller blebs that filtered more efficiently, it is possible that this altered inflammatory microenvironment resulted in decreased myofibroblastic transformation, which in turn led to decreased bleb contraction and secondary prolongation of stromal edema with improved bleb filtration capacity.

Overall, AgNPs represent a potential alternative to MMC as adjunctive therapy during filtration surgery and may be superior to MMC. While there is a mild increase in lymphocyte proliferation associated with AgNPs, they also lead to a sustained reduction of IOP and promote blebs with decreased fibrosis and ischemia as well as increased filtration. This combination of effects is ideal in filtration surgery, and may offer an opportunity to promote long-term surgical IOP reduction with an improved complication profile. Further study of the mechanisms governing the action of AgNPs on ocular tissue, the ideal delivery system for AgNPs, and long-term toxicity is warranted.

Acknowledgments

We thank Ronald L. Gross and Derek Nusbaum for critical reading of the manuscript. We thank Rebecca Penland (The Methodist Hospital Research Institute [TMHRI] Ocular Pathology Research Laboratory) for preparing and immunostaining the slides.

Supported by two Milton Boniuk, MD, Ophthalmology Resident Research Grants (MRB), the Endowment Fund of the Lions Eye

Bank Foundation (MRB), an unrestricted grant from Jamil Azzam, MD (SO-N), Research to Prevent Blindness, Inc. (BJF), National Institutes of Health (NIH) Grant K08 EY021479 (BJF), and NIH Grant P30 EY002520 (Baylor College of Medicine). The authors alone are responsible for the content and writing of the paper.

Disclosure: **M.R. Butler**, None; **C.M. Prospero Ponce**, None; **Y.E. Weinstock**, None; **S. Orengo-Nania**, None; **P. Chevez-Barrios**, None; **B.J. Frankfort**, None

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