

Original Article

Experimental investigation of needles, syringes and techniques for intravitreal injections

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ABSTRACT

Background: To assess the techniques and materials used in intravitreal injections.

Design: Descriptive study realized at the Vision Institute of the Federal University of São Paulo, Brazil.

Samples: Different brands of needles and syringes, as well as enucleated porcine eyeballs.

Methods: The ultra-structures of commonly used needles were analysed by scanning electron microscope, and they were compared using different criteria, such as irregularities and debris from the lubrication process. The scleral incision was also assessed using needles of different brands and sizes. Accuracies in drug administration were studied by comparing the residual and delivered volume of needles and also by the analysis of reflux after intravitreal injections.

Main Outcome Measures: Efficiency and quality of materials used in intravitreal injections.

Results: Ultra-structure analyses showed that all needles had different types of irregularities. Some photographs showed debris from the lubrication process, especially in BD needles. Scleral incision analysis showed a tendency of reducing the ocular damage with increasing gauge ($P = 0.024$). The investigation of delivery accuracy showed that almost all needles underdosed the amount injected ($P < 0.05$), and that the reflux could be minimized by tunnelled injections with thinner needles.

Conclusion: Needles used in intravitreal injections possess many irregularities in their structures, which may cause different injection outcomes. Analyses of scleral incisions showed that the larger the needle gauge, the lesser the scleral damage and the risk of complications. Moreover, drug administration inaccuracies might be one of the causes for some unsuccessful attempts of treatment.

Key words: intravitreal injection, needle, retinal pharmacotherapy, vitreal reflux, vitreoretinal surgery.

INTRODUCTION

Intravitreal injection (IVI) has been used in the management of human ocular diseases for nearly a century.^{1,2} Initially, in 1911, it was used to introduce air inside the intraocular space to repair retinal detachment. Recently, IVI became the mode of administration of two notable types of drugs capable of treating retinal diseases: (i) corticosteroids, such as triamcinolone acetonide, a longer-acting corticosteroid for the treatment of different types of macular oedema; and (ii) vascular endothelial growth factor inhibitors such as ranibizumab (Genentech, San Francisco, CA, USA) and bevacizumab (Genentech, San Francisco, CA, USA), which are efficacious and well-tolerated drugs in the treatment of patients with age-related macular disease, diabetic retinopathy and venous occlusions, among other diseases.^{3,4} As several new ophthalmological therapies are being investigated in clinical trials, the number of new IVI agents available should rise rapidly.

Thus far, there have been few studies on materials, techniques, needles and syringe sizes used in IVI.^{5–8}

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As the procedure may induce severe adverse events such as endophthalmitis or retinal detachment, appropriate technique and adequate monitoring are essential to reduce these sight-threatening complications, which may lead to permanent vision loss.^{9–11} In addition, patient discomfort as well as intraocular inflammation may be caused by uncontrolled techniques and materials.^{12,13} We have recently demonstrated that tunnelled scleral incisions significantly reduced vitreal reflux when compared with perpendicular incisions.¹⁴ Needle diameter has also been an important factor to be considered, as smaller caliber needles such as 30 and 31 gauge may cause less pain and smaller tissue injury than others.^{15,16} There may be additional factors such as the shape of needles, which may influence the pressure of scleral penetration and patient discomfort after IVI.^{17,18} Besides, the volume retained inside the needle must be considered, as the volume of the injected solution may be less than the desired volume, which may lead to therapeutic failures or toxicity.^{19–21} Considering the therapeutic importance, the diversity of available materials and the several ocular reactions that may occur, it is of utmost importance to have a detailed study about the techniques and materials used in IVI.

The purpose of the present study was to analyse these issues in IVI: (i) the ultra-structure of the needles used; (ii) the morphologic effects of scleral incisions with different needles in animal eyeballs; and (iii) drug delivery accuracies, assessed by residual and delivered volume (DV) in the syringes or by vitreal reflux after IVI.

METHODS

Ultra-structure analyses

The ultra-structural analyses were performed on 12 needles of five different brands: BD (Becton, Dickinson and Co., Franklin Lakes, NJ, USA), Terumo (Terumo Inc., Tokyo, Japan), KDL (KDL, Pacoima, CA, USA), Unijet (Plascalp, BA, Brazil) and Nipro (Nipro Inc., Osaka, Japan). These needles ranged from 26 to 31 gauge and were 8, 12.7 and 13 mm, which were selected as follows: BD 27G 13 mm, BD 28G 12.7 mm, BD 29G 12.7 mm, BD 30G 8 mm, BD 30G 12.7 mm, BD 31G 8 mm, KDL 26G 13 mm, Nipro 29G 13 mm, Terumo 26G 13 mm, Terumo 27G 13 mm, Terumo 29G 13 mm and Unijet 26G 13 mm.

In order to perform conventional imaging by scanning electron microscope, specimens must be electrically conductive at the surface. Nearly all of the needles used for IVI are coated with a thin layer of silicone to make the penetration easier and less painful for the patient. However, silicone interferes with the microscope imaging process, thus requiring

a gold deposition by the sputtering coating method, which is used in different situations regarding selected specimens and does not interfere with the imaging process. The chosen needles had their hub segmented and unattached from their syringes, without tampering with the area of study, and were coated with such gold layer on their surfaces.

The photographs were taken with a Jeol 3500 scanning electron microscope (Jeol Ltd., Tokyo, Japan) at four different angles: whole bevel vision (WBV), bevel apex (BA), bevel base (BB) and hub segment (HS). WBV photographs were taken to obtain a more general view of the bevel, without many details; the purpose of BA photographs was to analyse the point of the needle (i.e. the angle formed between the upper and lower 'distal' edges of the needles); BB images illustrate the initial portion of the needle, where the orifice of the bevel begins. Along with BA photographs, the flat side of the bevel can be seen in details; finally, HS photographs analysed the internal segment of the needles, the first part that a substance reaches after being ejected from the syringe. The photographs were analysed by two examiners to look for various ultra-structural characteristics: form of irregularities on surface, number of irregularities on surface, sharpness of tip, number of edges at bevel and debris from the lubrication process.

Scleral incisions study

The study of scleral incisions in enucleated pig eyes was performed with six different new needles: BD 28G 12.7 mm, BD 29G 12.7 mm, BD 30G 12.7 mm, BD 30G 8 mm, BD 31G 8 mm and Terumo 29G 13 mm needles. Two techniques of IVI were used: perpendicular and tunnelled incisions. For each type of injection, three procedures were carried out for each kind of needle, totalling 36 injections, each one of them being performed with a new needle. Tunnelled injections consisted in placing the needle through one-movement insertion in a 30-degree angle in a limbus parallel fashion 3.5 mm behind the limbus in the sclera. The angle of incision was standardized by the examiners, in order to minimize possible measurement variations.

Each eye was injected with 0.1 mL of trypan blue (TB) 0.1%, used to enable visualization of the incision site. In view of its larger diameter, the injection orifice was measured in its longest diameter with a scaled stereomicroscope (Leica MZ8; Leica Microsystems, Wetzlar, Germany), in which one unit corresponded to 400 µm. All the procedures were done by the same examiner. The results presented for each needle and type of injection represent the median of the three measurements. The collected data were analysed using the Wilcoxon non-parametric statistic test.

Residual and DV

The residual volume (RV) after IVI was assessed using five different pairs of BD syringe/needles: 28G 12.7 mm 0.3 mL, 29G 12.7 mm 1 mL, 30G 12.7 mm 0.3 mL, 30G 8 mm 1 mL and 31G 8 mm 0.5 mL. Initially, the pairs were weighed totally empty and filled with 0.1 mL of distilled water, that is, the 0.1 mL mark of each syringe. After being totally emptied, they were weighed once more to assess the RV. Thereafter, new pairs of the same BD syringe/needles were filled with 0.1 mL of distilled water and weighed, measuring the amount of DV and not the RV in the needle/syringe. Five measurements were made for each pair, in each procedure (totalling 10 measurements per group), and the actual volume injected (AVI) was then calculated by the following formula: $AVI = DV - RV$. The expected results were that if the confidence interval of 95% included the 0.1 mL value, it was possible that the needle/syringe in question injected the exact volume intended by the ophthalmologist. Otherwise, it could be considered an underdose or overdose, depending on the results obtained.

The measurements were done with a high-precision balance, Ohaus AS-200 (Ohaus, Pine Brook, NJ, USA). The results can be interpreted in mg or μ L, as all the needles were filled with distilled water, in which the density is 1 g/mL. The groups were statically compared using a *t*-test, with a significance level of 0.05. All these procedures were performed by the same examiner, and each needle/syringe pair was used only once.

Reflux after IVI

To analyse the amount and composition of the reflux after IVI, 0.1-mL TB (0.5 mg/mL) was injected in enucleated pig eyes using BD 27-, 30- and 31-gauge needles. Tunnelled and perpendicular incisions were made. Three injections were performed for each combination of needle gauge and incision type (tunnelled or perpendicular), totalling 18 injections, each one in a different eye. In a second set-up, enucleated bovine eyes were submitted to IVI injection of 0.2 mL of 0.5 mg/mL TB with either perpendicular or tunnelled incision, and with BD 27- or 30-gauge needles.

The amount of reflux was measured in its largest length, and a grading of 0 to 4+ (+++++) was used to describe the presence of TB in the reflux, where 0 was transparent fluid and 4+ dark bluish. All the procedures were carried out by the same examiner, and the colorimetric comparison was performed by three independent examiners.

RESULTS

Ultra-structure analyses

The analyses demonstrated different patterns among the needles assessed. Almost all of them showed certain irregularities on their surface. WBV photographs made it possible to evaluate the tip of the needles in a more general way, as different patterns were seen in the bevels. BD needles were the only ones to have three edges on their bevel, and all other brands had two edges. Regarding the straightness of the tips (i.e. the more 'distal' edge of the bevel), Terumo displayed more curved edges, and the tips were straighter in BD and Nipro; however, the straightest tips were observed with KDL and Unijet.

With BA photographs, one could observe that BD needles became more pointed as gauge increased. Terumo had needles with the roundest tips, whereas KDL had the most pointed ones. Nipro and KDL were more pointed than 31G BD, but less pointed than KDL.

In analysing BB photographs, along with BA photographs, a fixed pattern was determined for Terumo needles: the surface had a rough aspect in all its needles, among other irregularities. BD needles showed many kinds of irregularities, except for the 31G needle, which seemed to be the most regular of all of the needles assessed with this parameter. Nipro and Unijet both had longitudinal imperfections close to the tip, similar to striae. KDL also had some irregularities, especially along the edge of the bevel orifice.

In HS photographs, the surface of this portion of the needle was studied and the photographs showed that Nipro had the flattest surface, and BD demonstrated many irregularities, localized close to the proximal opening of the needle. Terumo also displayed some irregularities with a porous aspect.

Various photographs for each angle are shown in Figure 1.

Scleral incisions study

The results for the scleral incisions on pig eyes are summarized in Table 1. Comparing the scleral opening size after IVI, there is a tendency for reduced ocular damage with increase in gauge, in both types of injection. Statistical analysis of data between perpendicular and tunnelled incisions shows higher probability of scleral opening reduction when the latter technique is used.

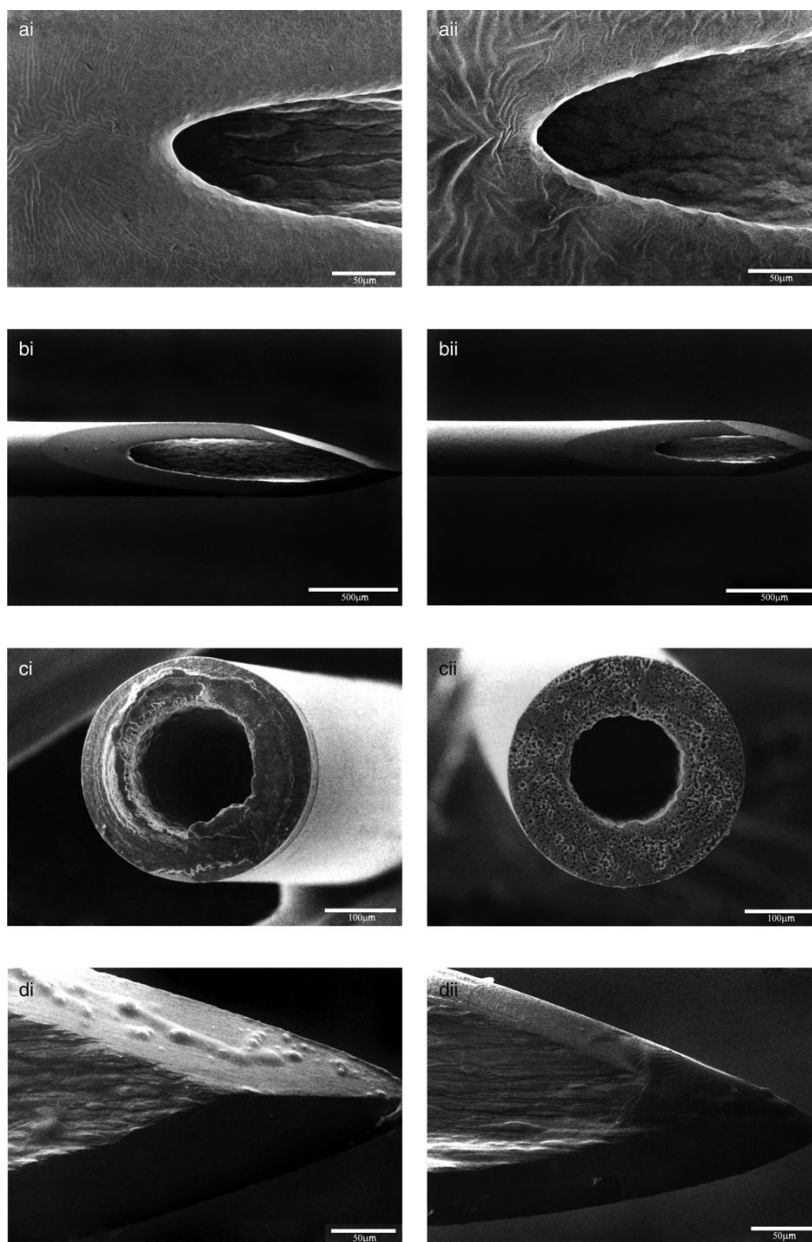
Residual and DV

The RV was the first measurement analysed. The results are described in Table 2. The RV analysis

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Figure 1. Scanning electron microscopic images of assessed needles. (a) Bevel base images (I: Nipro 29G 13 mm; II: Terumo 27G 13 mm); (b) whole bevel vision images (I: KDL 26G 13 mm; II: Terumo 29G 13 mm); (c) hub segment photographs (I: BD 29G 12.7 mm; II: Terumo 29G 13 mm); (d) bevel apex images (I: BD 27G 13 mm; II: BD 31G 8 mm).



showed that the group 28G 12.7 mm 0.3 mL induced the smallest volume ($0.62 \mu\text{L} \pm 0.15$), and the 30G 8 mm 1 mL produced the largest RV ($6.80 \mu\text{L} \pm 0.39$) ($P < 0.001$). After observing the RV, DV was assessed in a similar way. The results are shown in Table 3.

Using the available RV and DV results, it was possible to calculate the AVI, employing the formula described earlier. The final results are summarized in Table 4, which show that only the BD 30G 12.7 mm 0.3 mL group injected the exact amount intended. All other pairs underdosed the amount injected.

Reflux after IVI

The results of the colour and the largest length of reflux fluid investigated in porcine eyes are shown in Table 5. In both pig and bovine eyes, reflux occurred less often as the gauge increased and with tunnelled injections.

DISCUSSION

One of the objectives of this study was to investigate the ultra-structure characteristics of needles

commonly used for IVIs, as the ultra-structure of needles used for IVI varies from one brand to another. Twelve needles of five different brands were assessed, which ranged from 26 to 31 gauge and were 8, 12.7 and 13 mm. The surface of the needles showed remarkable irregularities and debris, which may interfere with scleral penetration. In addition, the surface of the bevel had rough patterns, and bevel angle and shape varied substantially. Potential clinical implications of such needle irregularities are intraocular inflammation, damage to the scleral tissue with consequent predisposition to vitreal reflux and additional patient discomfort.

The eye is a closed system and is sensitive to changes in intraocular pressure; therefore, drugs

must be administered by injection of small volumes of solution.²² The amount of drug injected into the eye is the product of the concentration and volume of the injected solution. Deviations from the intended quantities of these two factors may lead to therapeutic failures. Usual clinical practice for the therapy of neovascular, oedematous or inflammatory diseases is to deliver the desired dose of drug in 0.05- to 0.15-mL solutions.⁴ Few investigations have addressed the precision of the delivery of small volumes used in IVI. An experimental laboratory investigation performed in 2002 using 20 different syringes showed that the mean DV for each syringe ranged from 0.0884 to 0.0944 mL when the intended volume was 0.10 mL and from 0.0409 to 0.0451 mL when the intended volume was 0.05 mL, which was considerably less than the intended volume of injection. In that study, the mean 0.1- and 0.05-mL injection was 92% and 87% of the target volume, respectively. They also found overdelivery of volume to be infrequent and of small magnitude.²¹ Our results confirmed in part the inaccurate nature of the current syringes used for IVI; we found that the DV in most syringes was very inaccurate. On the other hand, the actual DV was often overestimated. In addition, our investigation is the first one to compare the results among different sizes and brands of syringes. We found that the RV was lower in small syringes of 0.3 mL connected to long 12.7-mm needles. These results imply that the usual 1-mL syringes are not the most appropriate ones for IVIs. Further studies should explore the clinical relevance

Table 1. Size of scleral hole after IVI, according to type of injection

Needle	Type of injection	
	Perpendicular	Tunnelled
BD 28G 12.7 mm	280	240
BD 29G 12.7 mm	220	180
BD 30G 12.7 mm	240	140
BD 30G 8 mm	220	160
BD 31G 8 mm	180	140
Terumo 29G 13 mm	260	220
Mean $\pm$ SD	233 $\pm$ 35	180 $\pm$ 42
Perpendicular vs. tunnelled	$P = 0.024^*$	

*Wilcoxon non-parametric statistic test. IVI, intravitreal injection.

Table 2. Results and comparisons of residual volume between needle/syringes analysed

Syringe + needle pair	Residual volume $\pm$ SD (mg)	P (t-test, sig $P < 0.05$)				
		1	2	3	4	5
1. BD 28G $\times$ 12.7 mm $\times$ 0.3 mL	0.62 $\pm$ 0.15	†				
2. BD 29G $\times$ 12.7 mm $\times$ 1 mL	5.08 $\pm$ 0.37	<0.001	†			
3. BD 30G $\times$ 12.7 mm $\times$ 0.3 mL	0.62 $\pm$ 0.19	1.00	<0.001	†		
4. BD 30G $\times$ 8 mm $\times$ 1 mL	6.80 $\pm$ 0.39	<0.001	<0.001	<0.001	†	
5. BD 31G $\times$ 8 mm $\times$ 0.5 mL	5.38 $\pm$ 0.10	<0.001	0.07	<0.001	<0.001	†

†Not applicable.

Table 3. Results and comparisons of delivered volume between needle/syringes analysed

Syringe + needle pair	Delivered volume $\pm$ SD (mg)	P (t-test, sig $P < 0.05$)				
		1	2	3	4	5
1. BD 28G $\times$ 12.7 mm $\times$ 0.3 mL	99.3 $\pm$ 0.3	†				
2. BD 29G $\times$ 12.7 mm $\times$ 1 mL	98.9 $\pm$ 0.4	0.338	†			
3. BD 30G $\times$ 12.7 mm $\times$ 0.3 mL	100.9 $\pm$ 0.8	0.038	0.023	†		
4. BD 30G $\times$ 8 mm $\times$ 1 mL	102.4 $\pm$ 0.3	0.011	0.005	0.107	†	
5. BD 31G $\times$ 8 mm $\times$ 0.5 mL	99.5 $\pm$ 0.7	0.478	0.270	0.014	0.030	†

†Not applicable.

of the accuracy and precision of the volume of injected solution in clinical trials.

Herein, the scleral incisions were investigated in porcine eyes with various sizes of needles and incision techniques. The techniques evaluated simulate those used for IVI in clinical practice. The results showed less ocular damage with increase in gauge with both tunnelled and perpendicular injections. In addition, lower risk of scleral damage was encountered with the tunnelled incision. These data are important, because a poorly formed scleral incision may influence the effectiveness of the injection and cause vitreous incarceration. The ensuing vitreous incarceration may increase the risk of retinal detachment or cystoid macular oedema. Moreover, inflammation or endophthalmitis could result due to a vitreous wick at the incision site. Regarding vitreous incarceration, an interesting hypothesis is that 31-gauge needles might cause less incarceration than the 30-gauge or 27-gauge needles. This finding is consistent because the injection wound from the thinner needle is much smaller than that of a wider one, which does not allow as much formed vitreous

to exit the globe. A smaller scleral wound acts more as a barrier to the vitreous than to the liquid drug. In the surgical entry site studies, the vitreous wick is not truly present at the ocular surface because the conjunctiva is closed, so the risk of endophthalmitis may be minimized. Future examination should clarify the dynamics of the drug in the vitreous cavity and scleral incisions.

Drug reflux through the injection site may limit the therapeutic efficacy of medications delivered by IVIs. Boon *et al.* showed that 46% of patients had reflux after the first injection of bevacizumab.²³ Our study used porcine and bovine eye models to evaluate whether the vitreal reflux contains drug in its composition. In this set-up, IVI of 0.1 mL of TB using BD 27, 30, and 31 gauges as well as tunnelled and perpendicular incisions were performed. The results showed a blue-coloured nature of the reflux in most eyes, and more intense with straight and large incisions. Another study performed similar experiments with methylene blue and proposed that the amount of refluxed drug is around 10% of that injected. Those authors also observed less reflux with small 30-gauge needles in comparison with 27-gauge needles, although an even smaller needle of 32 gauge caused more reflux than the 30 gauge. They explained such finding being due to the lack of intravitreal space with very small needles, but other factors such as a counteract force should be considered as well.²⁴ Although the tunnelled incision allows single-movement insertion of the needle and accurate drug injection, the relationship between the reflux bleb and amount of drug volume loss remains to be determined.

Table 4. AVI, calculated with the formula $AVI = DV - RV$

Syringe/needle pair	AVI (mg)
1. BD 28G × 12.7 mm × 0.3 mL	98.09–99.27
2. BD 29G × 12.7 mm × 1 mL	92.86–94.78
3. BD 30G × 12.7 mm × 0.3 mL	99.04–101.52
4. BD 30G × 8 mm × 1 mL	94.72–96.48
5. BD 31G × 8 mm × 0.5 mL	93.1–95.14

AVI, actual volume injected; DV, delivered volume; RV, residual volume.

Table 5. Analysis of reflux in pig eyes

Needle and type of injection			Grade of dye colouring	Length of episcleral bleb (mm)
27G needle	Non-bevelled	$n = 1$	+++	20
		$n = 2$	++	8
		$n = 3$	+++	2.5
	Bevelled	$n = 1$	++	9
		$n = 2$	+++	11
		$n = 3$	+	8
	Non-bevelled	$n = 1$	+++	4.5
		$n = 2$	++	8
		$n = 3$	++	4
30G needle	Non-bevelled	$n = 1$	++	4
		$n = 2$	++	<0.5
		$n = 3$	NA	0
	Bevelled	$n = 1$	+	2
		$n = 2$	++	<0.5
		$n = 3$	+	1
	Non-bevelled	$n = 1$	NA	0
		$n = 2$	+	<0.5
		$n = 3$	+	1

0, transparent; +, light bluish; ++, moderate bluish; +++, dark bluish; +++, very dark bluish as trypan blue. NA, not applicable.

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There are limitations in our study, which include the difference between human and animal vitreous, lack of quantification of vitreous reflux and lack of identification of the composition of the debris on the needle surface. We are planning to submit the needles for biochemical analyses to determine if the debris is composed of silicone, ions, metal or any other substance. Our study group also aims to examine the amount of vitreous after IVIs, thereby trying to quantify how much drug is lost after injection. Pig eyes are often and widely used for ophthalmic surgical training because of their anatomic similarities to human eyes.

The availability of new drugs may promote an increased use of IVIs in ophthalmology in the future. Understanding the flow of the drug inside the globe after the different techniques of IVI injection can provide important implications for clinical practice, given the small amount of drug used in IVI injections. In addition, minimizing vitreous incarceration may help to decrease the rate of serious complications. Although our investigation reveals potential systematic errors in the methods we employed, clinical experiences have shown that adequate safety and efficacy have been achieved in many circumstances using similar techniques. Further investigation should examine, through imaging evaluations, the scleral incision during IVIs, as well as individual risk factors for reflux, such as scleral stiffness.

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