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# EVALUATION OF SURGICAL FACTORS AFFECTING VITREOUS HEMORRHAGE FOLLOWING PORT DELIVERY SYSTEM WITH RANIBIZUMAB IMPLANT INSERTION IN A MINIPIG MODEL

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**Purpose:** To develop an animal model of vitreous hemorrhage (VH) to explore the impact of surgical parameters on VH associated with insertion of the Port Delivery System with ranibizumab (PDS) implant.

**Methods:** Ninety eyes from 45 treatment-naive male Yucatan minipigs received PDS implant insertion or a sham procedure. The effect of prophylactic pars plana hemostasis, scleral incision length, scleral cauterization, surgical blade type/size, and viscoelastic usage on postsurgical VH was investigated.

**Results:** Postsurgical VH was detected in 60.0% (54/90) of implanted eyes. A systematic effect on VH was only detected for pars plana hemostasis before the pars plana incision. The percentage of eyes with VH was 96.6% (28/29) among eyes that did not receive prophylactic pars plana hemostasis and 42.4% (24/58) among eyes that did. There was no VH in eyes that received laser ablation of the pars plana using overlapping 1,000-ms spots; pars plana cautery or diathermy was less effective. The majority of all VH cases (83.3% [45/54]) were of mild to moderate severity (involving ≤25% of the fundus).

**Conclusion:** In this minipig surgical model of VH, scleral dissection followed by pars plana laser ablation before pars plana incision most effectively mitigated VH secondary to PDS implant insertion.

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The availability of anti–vascular endothelial growth factor therapies specifically developed for intravitreal use, such as ranibizumab (Lucentis; Genentech, Inc, South San Francisco, CA) and aflibercept (EYLEA; Regeneron Pharmaceuticals, Inc, Tarrytown, NY), has had a profound impact on the management of multiple sight-threatening retinal diseases. However, optimal treatment requires frequent office monitoring and repeated intravitreal injections (as often as once monthly),[1–3] which are burdensome for patients, their caregivers, and health care providers.[4–7] As a result, office visits and injections in many clinical care settings are less frequent than those seen in pivotal clinical trials, and the visual improvements are neither as great nor as well maintained over time.[8–14] For this reason, new long-acting treatment strategies are needed to prolong intravitreal vascular endothelial growth factor suppression.

The Port Delivery System with ranibizumab (PDS) is an innovative, investigational drug delivery system designed to address the need for a less burdensome anti–vascular endothelial growth factor treatment paradigm by maintaining therapeutic drug concentrations in the vitreous for extended periods of time. The PDS includes a permanent, refillable intraocular implant that is surgically inserted through the sclera at the pars plana. A self-sealing septum in the center of the implant flange remains accessible through the conjunctiva and allows access to the implant reservoir for drug replenishment without the need to remove the implant from the eye. Ranibizumab moves by passive diffusion through a concentration gradient from the implant

reservoir through a release control element specifically designed for ranibizumab, providing continuous release into the vitreous cavity.

The safety and clinical potential of the PDS has been evaluated in an open-label Phase 1 study (NCT01186432)[15] and a randomized, multicenter, active treatment–controlled, dose-ranging Phase 2 clinical trial (Ladder; NCT02510794).[16] In the Phase 1 study, 5 of 20 participants experienced vitreous hemorrhage (VH) associated with the surgical procedures for PDS implant insertion, and this VH was accompanied by a transient, but in some cases prolonged, decrease in vision. The source of intraocular bleeding leading to VH after PDS implant insertion was unknown.

The Phase 2 Ladder trial was initiated in September 2015 to evaluate the safety and efficacy of the PDS compared with monthly intravitreal ranibizumab injections in patients with neovascular age-related macular degeneration responsive to anti–vascular endothelial growth factor therapy. During the first few months after study initiation, an unexpectedly high rate of postsurgical VH was observed in patients who received implants. This prompted a systematic evaluation of the effect of various surgical parameters on the risk of implant insertion–related VH to inform the conduct of the Phase 2 clinical trial.

The purpose of this study was to develop an animal surgical model of VH in Yucatan minipigs to explore the impact of various surgical parameters on VH associated with the PDS implant insertion procedure and to evaluate various techniques for mitigating VH.

## Methods

### *Experimental Animals*

Animal care and all procedures conducted in animals complied with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare, and all relevant local, state, and federal laws. The local animal care committee (Covance Laboratories, Inc, Princeton, NJ), in accordance with the Association for Research in Vision and Ophthalmology's Statement for the Use of Animals in Ophthalmology and Vision Research, approved all animal protocols.

A total of 45 treatment-naive male Yucatan minipigs (Sinclair Research Center, Inc, Auxvasse, MO) ≥2 months of age and weighing 8 kg to 16 kg were used. Animals were individually housed in stainless steel cages in an environmentally controlled room. They were provided with a certified diet (#5K99; PMI LabDiet, St. Louis, MO) and water ad libitum (fresh daily), along with supplemental dietary enrichment and cage enrichment devices.

### *Study Design*

The PDS implant was surgically inserted on Day 1, and animals were observed through Day 8 (when terminal necropsy was performed). The first 3 animals (6 eyes) received PDS implant insertion according to the surgical procedures described below. The surgical techniques used with the remaining 42 animals were then varied to explore the effect of prophylactic pars plana hemostasis, scleral incision length, scleral cauterization, surgical blade type and size, and/or viscoelastic usage (Healon Viscoelastic Prefilled Syringe; Alcon, Inc, Fort Worth, TX) on the occurrence of VH. Observations from each animal were used to inform the choice of specific procedures for

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subsequent animals. In addition, three eyes from three different animals received a sham surgical procedure (all steps except insertion of the PDS implant). The number of animals and eyes in the resulting treatment groups is listed in Table 1.

*Surgical Procedures (Day 1)*

*Preoperative.* All animals were fasted before surgery. Animals were anesthetized with dexmedetomidine, midazolam, ketamine, and butorphanol, followed by intubation and inhalation anesthetic. Each eye was dilated with a mydriatic agent and instilled with a topical anesthetic (0.5% proparacaine). The eyelids were retracted with a wire speculum and the eye prepared for surgery using standard aseptic techniques.

*Standard PDS implant insertion.* The eye was retracted to expose the lateral limbal area. A limbal-based conjunctival flap was then created and any scleral bleeding controlled with diathermy heat cauterization. An incision was then made in the scleral wall parallel and 4 mm posterior to the limbus at 9 o'clock in right eyes and 3 o'clock in left eyes. The incision was performed either as a full-thickness stab incision through sclera and pars plana or as a 3.2-mm to 4.1-mm dissection through the sclera to expose the underlying pars plana as designated in the protocol for that animal. The knives used for the scleral stab incision or dissection included the 3.2-mm Xstar slit knife, the 3.5-mm intraocular lens knife, the 4.1-mm implant knife, and the 0.9-mm (20 G) MicroVitreoRetinal knife (all from Beaver-Visitec International, Waltham, MA).

If prophylactic pars plana hemostasis was to be used, it was performed using cautery, laser ablation, or fine-tip bipolar pencil diathermy. Laser ablation was performed using a 532-nm green endolaser probe starting with 200-mW power and 200-ms to 1,000-ms laser burns. Overlapping burns were delivered to the pars plana ab externo, proceeding methodically from one edge of the incision to the other and then returning to the initiation

Table 1. Rate of VH by Type of Hemostasis and Type of Blade Used for the Scleral Dissection

| Treatment Group | No. of Animals | No. of Eyes | Eyes With VH, n (%) | Eyes With VH Extent Grade ≥3, n (%) |
|---|---|---|---|---|
| Sham surgical procedure (no implant) | 3 | 3 | 2 (66.7) | 0 |
| Scleral cautery only | 16 | 29 | 28 (96.6) | 9 (31.0) |
| Scleral cautery and prophylactic pars plana hemostasis | | | | |
| Pars plana cautery | 6 | 11 | 2 (18.2) | 0 |
| Laser ablation | | | | |
| Full field | | | | |
| (Laser Group A) 1,000 ms, PPI < SDL, straight blade for SD* | 4 | 8 | 0 | 0 |
| (Laser Group B) 1,000 ms, PPI > SDL, straight blade for SD† | 2 | 3 | 2 (66.7) | 0 |
| (Laser Group C) 1,000 ms, PPI < SDL, crescent blade for SD‡ | 3 | 3 | 2 (66.7) | 0 |
| (Laser Group D) <1,000 ms, PPI < SDL, straight blade for SD† | 9 | 15 | 12 (80.0) | 1 (6.7) |
| Corners only | | | | |
| (Laser Group E) <1,000 ms, PPI < SDL, straight blade for SD† | 3 | 3 | 1 (33.3) | 0 |
| (Laser Group F) 1,000 ms, PPI < SDL, crescent blade for SD§ | 3 | 3 | 1 (33.3) | 0 |
| Fine-tip diathermy§ | 6 | 12 | 6 (50.0) | 3 (25.0) |
| Total with pars plana hemostasis | 26¶ | 58 | 24 (42.4) | 4 (6.9) |
| Total receiving implants | 42¶ | 87 | 52 (59.8) | 9 (10.3) |
| Overall total | 45¶ | 90 | 54 (60.0) | 9 (10.0) |

*SD knife: 3.2-mm Xstar slit knife or 0.9-mm (20 G) MicroVitreoRetinal (MVR) blade. PPI knife: 3.2-mm Xstar slit knife or 3.5-mm BVI intraocular lens knife.

†SD knife: 0.9-mm (20 G) BVI MVR knife. PPI knife: 3.2-mm Xstar slit knife or 3.5-mm intraocular lens knife.

‡SD knife: 4.1-mm implant knife. PPI knife: 3.2-mm Xstar slit knife.

§SD knife: 4.1-mm implant knife or 0.9-mm (20 G) MVR blade. PPI knife: 3.2-mm Xstar slit knife.

¶The total number of animals is not equal to the sum of animals in the different groups because the right and left eyes were treated differently in some animals.

All knives from Beaver-Visitec International, Waltham, MA.

site, until shrinking and thinning of the pars plana was observed, with possible transudation of vitreous fluid. A stab incision was then made in the treated pars plana using a 3.2-mm Xstar slit knife or a 3.5-mm intraocular lens knife. Eyes treated with pars plana laser ablation were grouped for analysis on the basis of the extent of ablation of the exposed pars plana (full length or at the corners only), the scleral dissection length compared with the pars plana incision, and the type of blade (straight or crescent) used for the scleral dissection (Table 1, Laser Groups A–F).

The PDS implant was then inserted through the incision using a specially designed PDS insertion tool. The PDS implant and proprietary ancillary devices were provided by the study sponsor (Genentech, Inc). The PDS implants used were the same as those designed for the Phase 2 (Ladder) PDS clinical trial.

Because the PDS implant is not optimized for use in a minipig eye, it was secured in place using string sutures placed in the sclera to ensure proper seating in the scleral incision and that the implant remained in place. Before insertion, the PDS implant was filled with sterile balanced salt solution (BSS; Alcon Laboratories, Inc) using the initial fill needle. The conjunctival flap was closed using 7-0 Vicryl sutures.

*Postoperative.* On recovery, each animal received topical ophthalmic triple antibiotic and steroid ointment, atropine drops or ointment, and systemic tramadol, carprofen, flunixin meglumine, meloxicam, and/or buprenorphine.

### *Experimental Modulation of Intraocular Pressure Intraoperatively*

To evaluate the impact of intraocular pressure (IOP) on elimination/reduction of VH, IOP was increased through infusion or bolus intravitreal administration of 20-$\mu$L to 100-$\mu$L BSS.

### *Evaluations and Follow-up*

Ophthalmic evaluations were conducted by a board-certified veterinary ophthalmologist on Days 1 (before, during, and after PDS implant insertion surgery), 2, 3 or 4, 5 or 6, and 8. The adnexa and anterior portion of each eye were examined using a hand-held slit-lamp biomicroscope. The ocular fundus of each eye was examined using an indirect ophthalmoscope. Eyes were dilated with a mydriatic agent (e.g., 1% tropicamide) before examination with the indirect ophthalmoscope. Intraocular pressure was measured using a Tono-Pen applanation tonometer (Reichert Technologies, Depew, NY), with three readings per eye collected at the time of ophthalmic evaluations. Ocular photographs were also taken using either a slit-lamp camera or a digital external hand-held camera.

Observed VH on indirect ophthalmoscopy was evaluated using a 6-point scale (from 0 = none to 5 = 76%–100% of fundus) to describe the extent of fundus involvement, and on a 4-point scale (from 0 = normal to 3 = severe) to describe the density of the hemorrhage (Table 2). After the ophthalmic examination and IOP measurement on Day 8, all animals were euthanized using sodium pentobarbital followed by exsanguination.

A subset of 30 eyes were collected for imaging by computed tomography (CT) to provide anatomical evidence to support the validity of the experimental model and to determine whether any bleeding could be detected that was not seen during the clinical examination. Briefly, the eyes were stained by immersion in a solution of metallic iodine (2% wt/wt) and potassium iodide (2% wt/wt) for 12 hours to 24 hours before scanning.[17] The globes were then rinsed in saline and the vitreous injected with saline to elevate the IOP to between 10 and 20 mmHg. Eyes were then wrapped in moist gauze and transferred to sealed polypropylene containers for CT scanning. Globes were scanned using a Hamamatsu x-ray source set at 150 KV and 115 $\mu$A, using an 80-ms integration time for each projection. Seven hundred and twenty angular projections were captured by a detector with 127-$\mu$m pixel pitch and a geometric magnification factor of ×4.5, yielding a CT voxel size of 28 $\mu$m.

### *Data Analysis*

Owing to the exploratory nature of the study design, the data are described using descriptive statistics only. Statistical tests of between-group differences were not conducted because the different groups of eyes often differed from each other in more than one surgical parameter.

## Results

A total of 90 eyes from 45 animals were included in this study. Of these, 87 eyes received a PDS implant insertion procedure and 3 received a sham surgical procedure (all steps except insertion of the PDS implant; Table 1).

### *Vitreous Hemorrhage on Ophthalmic Examination*

Vitreous hemorrhage of any severity at any time up to 8 days postoperatively was detected in 2 of 3 (66.7%) control eyes and 52 of 87 (59.8%) implanted eyes (Table 1). Most cases of VH (83.3% [45/54 eyes]) were mild to moderate in extent (involving

Table 2. Vitreous Hemorrhage Grading Scales Used in Minipig Model of the PDS Implant Insertion Procedure

| Grade | Description |
|---|---|
| Extent of involvement scale | |
| 0 | No hemorrhage present |
| 1 | Hemorrhage is limited to the surface of the PDS implant and extends no more than twice the width of the implant in the surrounding vitreous |
| 2 | Hemorrhage is greater than observed in grade 1 and involves ≤25% of the fundus |
| 3 | Hemorrhage involves 26%–50% of the fundus |
| 4 | Hemorrhage involves 51%–75% of the fundus |
| 5 | Hemorrhage involves 76%–100% of the fundus |
| Density of hemorrhage scale | |
| 0 | Normal—details of the underlying retina are clearly visible |
| 1 | Mild—details of the underlying retina are visible but slightly hazy |
| 2 | Moderate—details of the underlying retina are not visible, but major structures such as retinal or choroidal blood vessels and the optic disk are discernible |
| 3 | Severe—the fundus is not visible through the hemorrhage |

PDS, Port Delivery System with ranibizumab.

≤25% of the fundus). The VH density grade for most cases was two or three (moderate to severe), consistent with a localized hemorrhage in the area of the PDS implant that had not dispersed throughout the vitreous.

The surgical parameter that had the greatest impact on the incidence of VH in this animal model was pars plana hemostasis after the scleral dissection and before the pars plana incision, and the type of pars plana hemostasis (Table 1). Other parameters such as incision length and blade type only had an impact on the occurrence of VH if used in conjunction with pars plana hemostasis.

In eyes treated with scleral cautery alone (no prophylactic pars plana hemostasis), 96.6% (28/29 eyes) experienced VH regardless of incision length, blade type, or any other surgical parameter (Table 1 and Figure 1). The occurrence of VH decreased to 42.4% (24/58) among eyes treated with any type of pars plana hemostasis before the pars plana incision. Among eyes that received prophylactic pars plana hemostasis, the lowest rate of VH (0 of 8 eyes) was seen in eyes that received laser ablation of the pars plana with overlapping 1,000-ms spots after a scleral dissection that exposed the full length of the intended pars plana incision (Table 1, Laser Group A). Any deviation from this procedure resulted in an increased rate of VH (Table 1). For example, the VH rate was 66.7% (2 of 3 eyes) if the pars plana stab incision exceeded the length of the scleral dissection, most likely because such incisions would cut through pars plana tissue that had not been exposed for laser ablation, as well as some scleral tissue (Table 1, Laser Group B). The rate was similarly high if a crescent blade was used for the scleral dissection (66.7% [2 of 3 eyes]), and this may have been because this blade creates a beveled edge at the corners that may have prevented adequate full-field laser ablation (Table 1, Laser Group C). The VH rate was particularly high (80.0% [12 of 15 eyes]) if the duration of laser spots was <1,000 ms, and this suggests that laser spots of shorter duration are unable to create adequate full-thickness ablation of the pars plana (Table 1, Laser Group D). Our results also show that applying the laser to just the corners of the exposed pars plana (Table 1, Laser Groups E and F) was not as effective as full-field laser ablation.

Fine-tip diathermy was not as effective as laser ablation (VH in 50.0% [6 of 12] eyes). Pars plana cautery was associated with a low rate of VH (18.2% [2 of 11 eyes]), but it was also associated with scleral retraction at the sclerotomy site, which is considered suboptimal when placing a long-term implant.

The extent, as well as incidence, of VH was also affected by pars plana hemostasis before the pars plana incision. The percentage of eyes with VH extent of grade ≥3 was 31.0% in eyes treated with scleral cautery alone, 25.0% in eyes treated with fine-tip diathermy, and 0.0% to 6.7% in eyes treated with pars plana laser ablation before the pars plana incision (Table 1).

Modulation of IOP had no clear effect on the incidence of VH. The use of a viscoelastic to facilitate insertion of the PDS implant did not seem to affect the incidence of VH, but may have contributed to a risk of implant extrusion during the implant insertion procedure.

### *Computed Tomography Imaging*

The 28-$\mu$m voxel size provided sufficient resolution to visualize and/or measure the cornea, anterior chamber depth, lens, vitreous, ciliary process, suspensory ligaments (Figure 2A), and tissue deformation at the implant insertion site (Figure 2, B and C).

The staining solution clearly marked intravitreal blood, allowing the location and size of VH to be identified (Figure 2D). Greater than 93% agreement was found when CT VH detection was compared with clinical slit-lamp examination results, with CT scans detecting some degree of VH in 2 additional eyes. In the instances where VH was detected only in the CT analysis, the volume of blood was small and located on the posterior side of the implant.

Asymmetrical scleral thickness was noted in the CT scans, with a mean scleral thickness of 1.76 mm anterior to the insertion site and 1.31 mm posterior to the insertion site (Table 3 and Figure 2E). The symmetrical scleral thickness typical in human eyes is shown in a CT scan of a cadaver globe in Figure 2F.

## Discussion

To the best of our knowledge, this study describes the first animal surgical model for VH. This model proved useful for the investigation of the insertion of long-acting, indwelling devices into the vitreous cavity and provided important information that may be relevant to similar surgical approaches in different settings.

There is a risk of intraocular bleeding after PDS implant insertion because the procedure requires a pars plana incision without wound suturing. In this animal study of the PDS implant insertion procedure, the pars plana was found to be the main source of intraocular bleeding in the postoperative period. The only surgical parameter that was consistently associated with mitigation of VH incidence and severity after PDS implant insertion was the use of pars plana hemostasis before the pars plana incision. The most effective method for mitigating VH was prophylactic hemostasis using laser ablation of the pars plana with overlapping 1,000-ms 532-nm laser spots after a scleral dissection that exposed the full length of the intended pars plana incision (see **Video, Supplemental Digital Content 1**, http://links.lww.com/IAE/B42, showing scleral dissection in the superotemporal quadrant of a minipig eye, with exposure of pars plana and laser ablation with overlapping laser spots). Other parameters, such as incision length and blade type, only had a positive impact on the occurrence of VH if used in conjunction with pars plana laser ablation and only if they impacted the thoroughness of the laser ablation. The high rate of VH among eyes treated with scleral cautery alone (no prophylactic pars plana hemostasis) suggests that the scleral blood vessels do not contribute to postoperative intravitreal bleeding in this model.

Full-thickness stab incisions through the sclera and pars plana to access the vitreous cavity are commonly performed to remove foreign bodies within the eye[18] or to insert large implants that are sutured to the sclera.[19,20] Vitreous hemorrhage can occur after large incisions of the uvea, but large incisions are not that common in clinical practice. In view of this, the occurrence of VH after PDS implant insertion in PDS clinical trials was unexpected. The pars plana of the uvea is a highly vascularized anatomical structure[21] that can easily bleed after large pars plana incisions. However, suturing these incisions reduces the risk of intraocular bleeding and VH by providing tissue reapproximation and compression that promote hemostasis. The PDS implant insertion procedure does not require wound suturing because the body of the implant permanently resides within the scleral wound, and the flange of the implant creates a sealing effect. Nonetheless, open edges of the pars

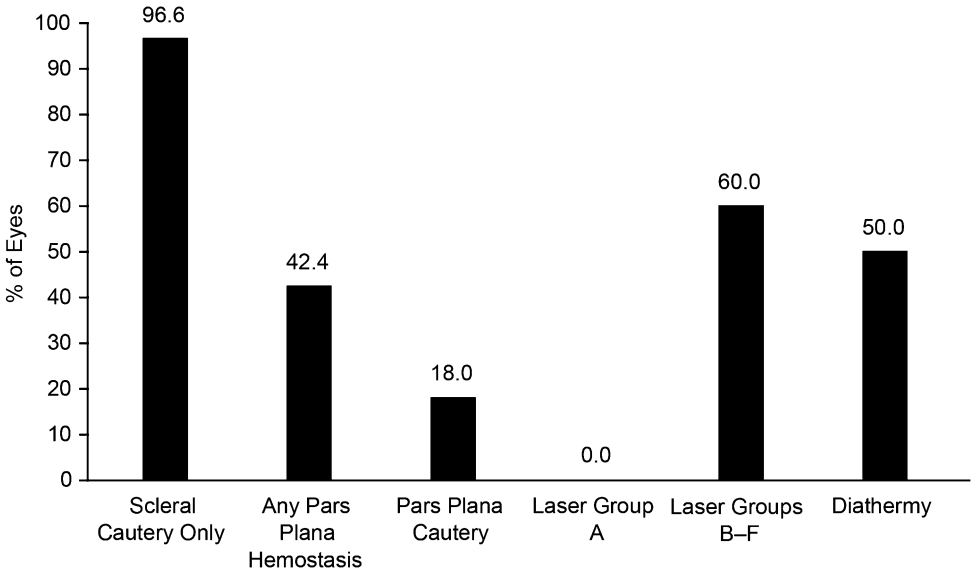


**Fig. 1.** Proportion of eyes with vitreous hemorrhage at any time during the first 8 days after implant insertion surgery. Laser Group A: 1,000-ms duration laser spots, full-field ablation, pars plana incision shorter than scleral dissection, straight blade used for scleral dissection. Laser Groups B–F: ≤1,000-ms duration laser spots and/or less than full-field ablation and/or pars plana incision shorter than scleral dissection, and/or crescent blade used for scleral dissection.

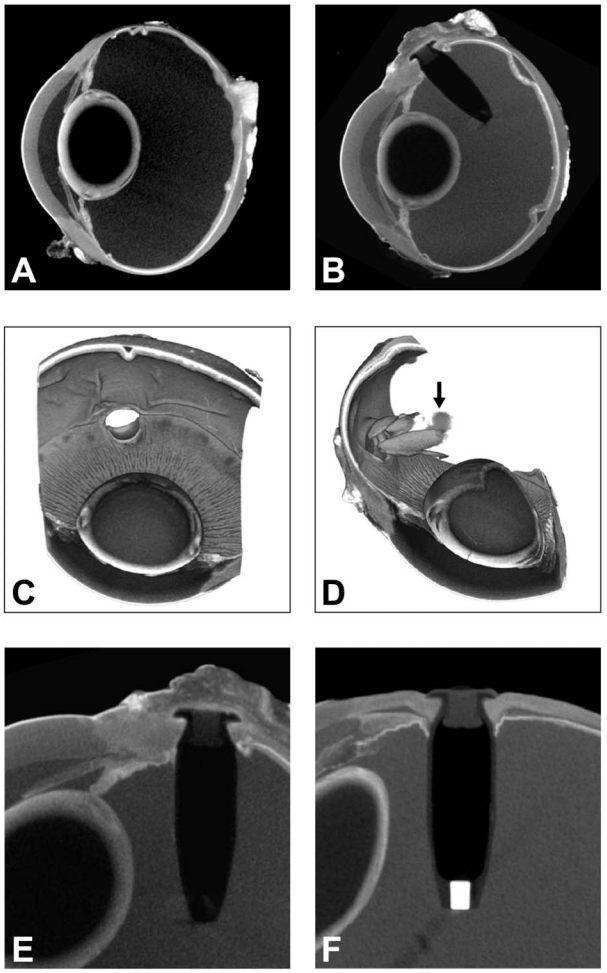


**Fig. 2.** Computed tomography images. **A.** Example of whole minipig globe showing cornea, anterior chamber, lens, and vitreous. **B.** Example of a whole minipig globe showing the Port Delivery System with ranibizumab (PDS) implant in place. **C.** Perspective view of the PDS implant insertion site in minipig globe. The PDS implant was digitally subtracted using image analysis software for clarity. **D.** Perspective view of the PDS implant insertion site in minipig globe with pars plana bleeding (arrow) that led to vitreous hemorrhage. The PDS implant was digitally subtracted for clarity. **E.** Magnified view of the PDS implant in a minipig eye, showing differential scleral thickness at the PDS implant insertion site. **F.** Magnified view of a PDS implant inserted into a human cadaver eye, showing symmetric scleral thickness at the insertion site. Cadaver globe does not include conjunctival tissue.

plana around the implant body could allow bleeding from severed vessels, eventually leading to VH.

Intraocular pressure modulation to increase pressure on the wound could be the first step to reduce intraocular bleeding, but is likely to be only a temporary solution. Indeed, as shown in this study, increasing IOP was not sufficient to mitigate VH.

Only prophylactic hemostasis with the 532-nm laser on the exposed pars plana mitigated VH. Moreover, we observed that a long laser duration was critical to reducing intraocular bleeding from the pars plana; 1,000-ms laser spots proved to be the most effective way to achieve full-thickness ablation of the pars plana (no VH was observed with this laser duration). Shorter duration (e.g., 200–500 ms) laser spots were not as effective at mitigating VH, most likely because they created only a superficial (partial-thickness) ablation. Finally, we demonstrated that a longer scleral dissection compared with the pars plana incision was critical to mitigate intraocular bleeding from the pars plana; by exposing and ablating an area of pars plana that was larger than the actual incision, we were able to avoid any VH because no untreated pars plana was incised.

Alternative tools commonly available for tissue hemostasis in vitreoretinal surgery are the scleral cautery tip and fine-tip diathermy. Although somewhat effective, neither of these alternatives was able to eliminate VH in this surgical model. The cautery tip, typically used for scleral hemostasis, is relatively large and not able to precisely burn the exposed pars plana because the scleral opening is quite tight. Moreover, the large cautery tip tends to affect the surrounding sclera more than does laser ablation, possibly resulting in distortion of the incision and poor fit of the implant. Fine-tip diathermy is known to be effective for performing localized evacuative punctures through the choroid to remove subretinal fluid ab externo, but it is not as effective in ablating full-thickness large choroidal areas. Moreover, with repeated touches of the pars plana, the diathermy tip can lose performance due to the accumulation of coagulated pars plana tissue, eventually leading to a nonhomogeneous hemostasis of the pars plana. The small laser burns and the precision of laser delivery, in contrast, allow for a more controlled and homogeneous hemostasis with minimal impact to the adjacent scleral wound edges. Overall, it seems that full-field laser ablation with overlapping 1,000-ms spots of a 532-nm laser was the most effective way to prevent VH because it allowed a precise, full-thickness ablation of the entire area of the pars plana incision with minimal damage to the surrounding sclera.

The appropriateness of this minipig model of VH is supported by the CT imaging results from this study. These high-resolution images revealed important ocular dimensions, confirmed that the overall structure of the minipig eye is suitable for surgical investigations of this type, and confirmed the VH results found on ophthalmic evaluation. This is important because, to the best of our knowledge, there was no established surgical model for VH at the time this study was initiated.

The results of this animal study were of immediate clinical importance to the Phase 2 clinical trial of the PDS in patients with neovascular age-related macular degeneration (Ladder) that was ongoing at the time of this animal study.[16] An unexpectedly high rate of VH

Table 3. Minipig Eye Anatomical Measurements Obtained Through CT Imaging

| Structure | Measurement, Mean ± Standard Deviation |
|---|---|
| Corneal thickness (n = 30), mm | 1.5 ± 0.1 |
| Anterior chamber | |
| Width (n = 30), mm | 11.2 ± 0.7 |
| Volume (n = 3), $\mu$L | 97.3 ± 19.8 |
| Lens dimensions | |
| Length (n = 30), mm | 7.0 ± 0.3 |
| Width (n = 30), mm | 9.1 ± 0.3 |
| Height (n = 30), mm | 8.9 ± 0.3 |
| Vitreous chamber volume (n = 3), $\mu$L | 2,170.6 ± 103.0 |
| Globe | |
| Length (n = 30), mm | 18.1 ± 0.6 |
| Width (n = 30), mm | 21.0 ± 0.7 |
| Height (n = 30), mm | 20.2 ± 0.8 |
| Volume (n = 30), $\mu$L | 3,781.9 ± 268.9 |
| Differential scleral thickness (n = 5) | |
| Anterior thickness, mm | 1.8 ± 0.3 |
| Posterior thickness, mm | 1.3 ± 0.1 |

CT, computed tomography.

was observed in the first 22 patients who received the PDS implant in that clinical trial. When the surgical protocols of the trial were modified based on the results of this animal study to include pars plana laser ablation, the rate of VH fell from 50% (11/22) to less than 5% (7/157) of PDS-treated patients. The optimized surgical protocol with pars plana laser ablation is currently being used in the ongoing Archway Phase 3 clinical trial of the PDS in neovascular age-related macular degeneration (ClinicalTrials.gov NCT03677934).

One limitation of this study was the small number of eyes in each experimental group and the fact that the different groups of eyes often differed from each other in more than one surgical parameter. Therefore, only descriptive summaries were provided. It should also be noted that the PDS implant is not optimized for use in the minipig eye. It should be noted that the minipig CT imaging indicated asymmetry of scleral thickness anterior and posterior to the implant that was not observed in human cadaver globes (Figure 2, E and F; Table 3). This asymmetry could have impaired proper implant seating and contributed to a high rate of VH in this animal model.

In conclusion, this surgical study in a minipig model of VH suggests that scleral dissection followed by thorough laser ablation of the pars plana before the pars plana incision is the most critical step in the reduction or elimination of VH during the placement of the PDS implant into the vitreous cavity. This discovery has been applied to clinical trials of the PDS, and the ability of the optimized surgical protocol to mitigate VH in human eyes receiving the PDS has been documented.[16]

**Key words:** vitreous hemorrhage, animal surgical model, Port Delivery System, anti–vascular endothelial growth factor, retinal disease.

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