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# Feasibility of Direct Vitrectomy-Sparing Subretinal Injection for Gene Delivery in Large Animals

**Left running head:** Z. STRANAK ET AL.

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## Footnotes

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## ABSTRACT

### Purpose

To assess the safety and feasibility of direct vitrectomy-sparing subretinal injection for gene delivery in a large animal model.

### Methods

The experimental Liběchov minipigs were used for subretinal delivery of a plasmid DNA vector (pS/MAR-CMV-copGFP) with cytomegalovirus (CMV) promoter, green fluorescent protein (GFP) reporter (copGFP) and a scaffold/matrix attachment region (S/MAR) sequence. The eyes were randomized to subretinal injection of the vector following pars plana vitrectomy (control group) or a direct injection without prior vitrectomy surgery (experimental group). Intra- and post-operative observations up to 30 days after surgery were compared.

### Results

Six eyes of three mini-pigs underwent surgery for delivery into the subretinal space. Two eyes in the control group were operated with a classical approach (lens-sparing vitrectomy and posterior hyaloid detachment). The other four eyes in the experimental group were injected directly with a subretinal cannula without vitrectomy surgery. No adverse events, such as endophthalmitis, retinal detachment and intraocular pressure elevation were observed post-operatively. The eyes in the experimental group had both shorter surgical time and recovery while achieving the same surgical goal.

### Conclusions

This pilot study demonstrates that successful subretinal delivery of gene therapy vectors is achievable using a direct injection without prior vitrectomy surgery.

## KEYWORDS

Subretinal injection; pars plana vitrectomy; large animal; non-viral gene vector; safety

## Funding

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## Introduction

A growing number of inherited retinal diseases (IRD) have been the target of preclinical and clinical research using ocular gene therapy. Gene replacement is a therapeutic approach that introduces wildtype cDNA into patient cells which can be transcribed and translated into normal protein, thus correcting genetic defects and improving cellular function.[1,2] Several types of vectors have been employed for gene delivery. Viral vectors (adeno-associated viruses, adenoviruses, retroviruses and lentiviruses) are modified viruses that can deliver genes to target cells efficiently. Non-viral vectors (liposomes, nanoparticles, and naked DNA) are less efficient than viral vectors but have the advantage of being safer and less immunogenic. In addition, cell-based vectors (stem cells or immune cells) can be used to deliver genes to specific tissues or organs and potentially provide long-term gene expression.[3,4]

Currently, adeno-associated viruses (AAV) remain the vector of choice for retinal gene therapy. They are the most efficient vectors to transduce retinal cells such as photoreceptor cells or retinal pigment epithelial (RPE) cells. In 2017, the US Food Drug Administration (FDA) approved Luxturna (Voretigene neparvovec) as the first retinal gene therapy for patients with confirmed biallelic RPE65 mutation-associated retinopathy.[5,6] Voretigene neparvovec uses an AAV2 vector and is injected subretinally. Although many clinical trials are underway, Luxturna has remained the only FDA approved gene therapy for IRD. Many promising clinical trials for IRDs, with similar AAV vectors were halted or did not meet primary or/and secondary endpoints.[7,8] It has been reported that AAV vectors can trigger harmful inflammatory responses and result in drug-induced chorioretinal atrophy, which has been noted widely amongst real-world patients treated with voretigene neparvovec.[9,10] Many aspects of retinal gene therapy safety were initially overlooked such as inflammatory side effects arising from the AAV capsid or its transgene, but this may also be increased or promoted by the injection routes and the surgical procedure [Please add full stop at the end of the sentence][11,12]

Alternatively, non-viral vectors (liposomes, nanoparticles, and naked DNA) are being developed. Synthetic vectors do not outperform AAV vectors yet, however they offer a less immunogenic strategy, potentially allowing for repeat administration.[13] Recent reports describe a promising plasmid DNA plasmid vector containing a Scaffold Matrix Attachment Region (S/MAR) sequence with a cytomegalovirus (CMV) or a CAG promoter for 2 different inherited retinal diseases.[14] S/MARs are AT-rich genomic motifs anchoring the

chromatin to the nuclear matrix, when incorporated with a transgene, they confer episomal maintenance, mitotic stability and prevent epigenetic silencing.[15–18]

Ocular gene therapy is dose and volume dependent, somewhat similar to pharmacological therapy. Hence, optimizing delivery, devising a reproducible, standardized method to appropriately target retinal tissues and layers can improve the experimental and clinical results by minimizing the loss of vectors from the intended target and reducing the risk of an immune response.[19,20] For diseases affecting photoreceptors and the retinal pigment epithelium (RPE), subretinal delivery has been considered a suitable approach for direct targeting of these retinal cell layers. Currently, subretinal injection delivery is preceded by pars plana vitrectomy (PPV) surgery with a posterior hyaloid detachment for creation of the bleb. All human trials up to date have used PPV prior to bleb formation.[21–24] While vitrectomy surgery is generally safe, it can be associated with complications such as postoperative cataract formation, infection, retinal detachment or increased intraocular pressure. Additionally, vitrectomy is an invasive surgical procedure requiring specialized equipment and expertise, limiting its availability and increasing the cost.

We hypothesize that an alternative simplified approach without vitrectomy surgery would be safer and faster with the same outcome of gene delivery. Additionaly, we believe it would reduce post-operative inflammation and accelerate surgical healing. Herein, we present an approach of direct vitrectomy-sparing subretinal injection to deliver a DNA S/MAR plasmid vector into the retina of a large animal model - the mini-pig. Its comparable size to the human eye, the presence of area centralis with highly concentrated cone photoreceptors, the well-described immune system, and the availability of methods to assess the morphology and function post-surgery make this an ideal model of large animal for the purpose of this study.[25,26]

## Materials and methods

### Animals

Large animal model: Libechov mini-pig.

The experimental herd of Liběchov minipigs was founded in 1967 by importing several animals from the Hormel strain from the USA. Animals were crossbred with several other breeds or strains: Landrace, Large White, Cornwall, Vietnamese pigs, and miniature pigs of Göttingen origin.[25,26] At 5 months of age and approximately 20 kg body weight (BW), the minipigs reach sexual maturity. The retina of minipigs lacks a macula and fovea. However, it has area centralis with highly concentrated cone photoreceptors. These regions are responsible for the highest visual acuity.

All experiments were carried out according to the Guidelines for the Care and Use of Laboratory Animals and according to the Association for Research in Vision and Ophthalmology (ARVO) for the use of animals in ophthalmic and visual research. The study protocol was approved by the Resort Professional Commission of the CAS for Approval of Projects of Experiments on Animals at the Institute of Animal Physiology and

Genetics of theCzech Academy of Sciences (Liběchov, Czech Republic) (Approved protocol No. 64/2022).

## Formulation of the DNA vector solution

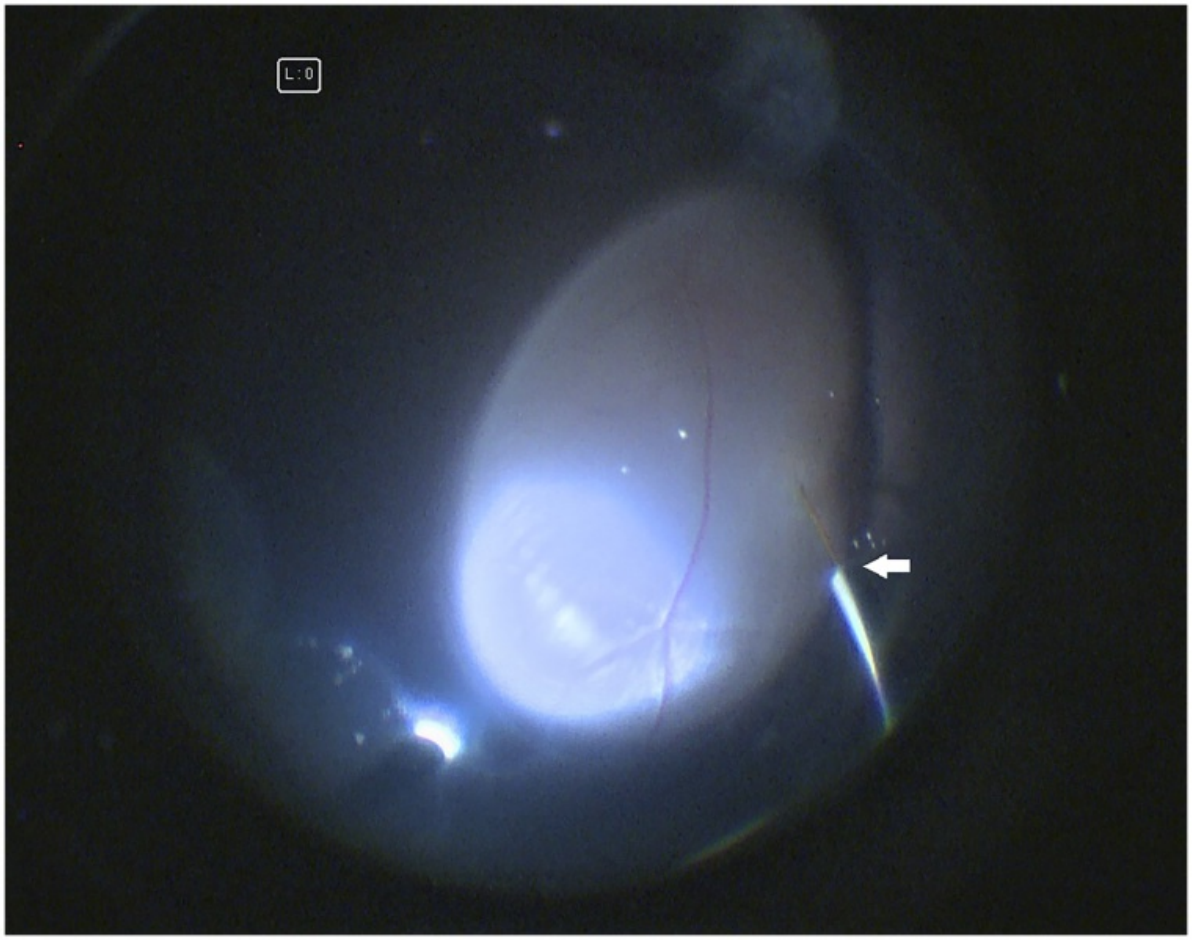
A plasmid DNA vector (pS/MAR-CMV-copGFP) with CMV promoter, GFP reporter (copGFP) and a scaffold/matrix attachment region (S/MAR) sequence was used for the mini-pig subretinal injections.[14] The plasmid was complexed with linear polyethylenimine (*in vivo*-jetPEI, Polyplus) in a 5% glucose solution at an N/P ratio of 8, following manufacturer's instructions. A final DNA concentration of 100 ng/µl in a total volume of 0.1 ml per bleb was injected into each eye.

## Anesthesia and surgical technique

The surgeries were performed by two experienced vitreoretinal surgeons (IK, ZS). In all cases, the surgery was performed under general anesthesia in an operating room with the standard aseptic and antiseptic measures. Specific conditions and surgical procedure are described previously by Lytvynchuk et al.[27] Briefly, we induced general anesthesia with an intramuscular injection of a mixture of tiletamine (2 mg/kg), zolazepam (2 mg/kg), ketamine (2 mg/kg), and xylazine (0.4 mg/kg) prior to the surgery. Then, we intubated the animal with an endotracheal tube for the inhalation maintenance of anesthesia (1.5% isoflurane) using an anesthesia machine equipped with a monitor. The mini-pig was shaved in the area around the eye and disinfected with a 10% povidone-iodine solution. The operating field was covered with a sterile ophthalmic drape, a lid speculum was inserted and lateral canthotomy done for better exposure.a/Pars plana vitrectomy - assisted subretinal injection

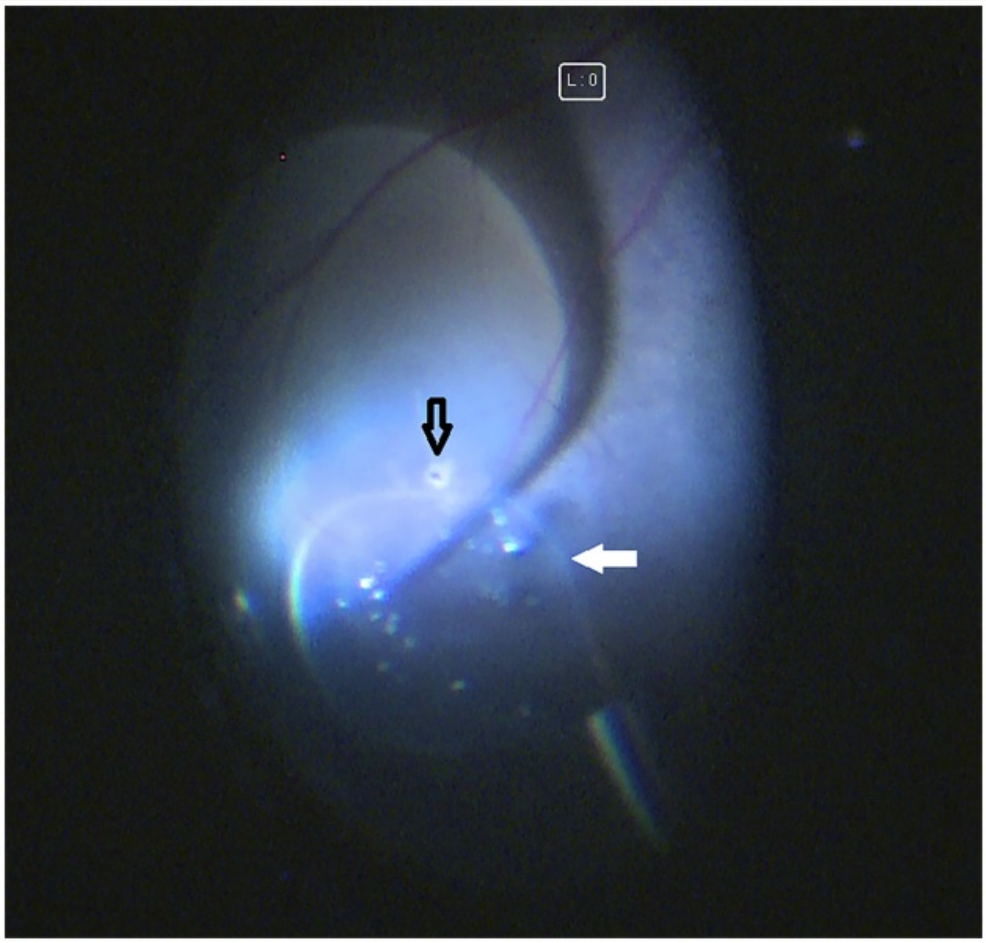
The ophthalmic surgery microscope Hi-R NEO 900 A (Haag-Streit, Switzerland) and R-Evolution CR (Optikon, Italy) vitrectomy machine were used for surgery. Vitrectomy surgery commenced with insertion of the three-valved 23 G trocars followed by standard lens-sparing PPV procedure with posterior hyaloid detachment (using triamcinolone acetonide for visualization) and hydraulic subretinal bleb formation with injection of 0.1 ml volume of the vector at two sites inside the posterior pole and along the vascular arcades using 41 G subretinal cannula (control group)(Figure 1). The retina was inspected for any pathology such as bleeding or peripheral tears. Finally, the 23 G trocars were removed and the sclerotomies were sutured with 8.0 Vicryl sutures. No intraocular tamponade was used. We applied topical antibiotic ointment and patched the eye. The animal was transferred to a recovery room.b/Direct PPV-sparing subretinal injection

Figure 1. Intraoperative fundus photo of pars plana vitrectomy assisted subretinal injection of non-viral gene vector in minipig model demonstrates a bleb formation using 41 G needle delivery (white arrow)(Please see Video 1).




In the vitrectomy-sparing (experimental) group, we inserted three-valved 23 G trocars and advanced directly to the subretinal injection of DNA vector (0.1 ml in volume) using a 23/41 G subretinal extendable cannula connected to a calibrated reservoir with the vector. We injected at the corresponding retinal locations similar to the control group. Hydraulic retinotomy was created by a stream of injection volume which could be seen before bleb rise (Figure 2). Following that, the trocars were removed and the sclerotomies sutured with 8.0 Vicryl sutures. We applied topical antibiotic ointment and patched the eye. The animal was transferred to a recovery room.

Figure 2. Intraoperative fundus photo of vitrectomy-sparing direct subretinal injection of non-viral gene vector in minipig model demonstrates a formed bleb with retinotomy (black arrow) and escaped injection solution before bleb formation (white arrow)(Please see Video 2).




## Post-op care/imaging

The ocular condition and subsequent well-being of the animals were monitored daily. A macroscopic evaluation of the eye and its surroundings was performed, food intake was recorded, and signs of ocular pain or irritation were assessed. Post-operatively, topical bacitracin zinc/hydrocortisone acetate/neomycin sulfate was applied into the conjunctival sac of the animals five times per day for 2 weeks. Anterior segment, fundoscopic examinations and intraocular pressure (IOP) measurements were performed under general sedation (intramuscular injection of tiletamine/ketamine mixture) on post-op days 3, 7, 16 and 30.

For *in vivo* imaging, the operated eyes were dilated using 0.5% tropicamide eye drops and first examined by indirect ophthalmosocopy. Color fundus images were taken with a color nonmydriatic fundus camera (TopCon, Japan). Additionally, we took a red-free image of the retina with the non-mydriatic fundus camera and performed optical coherence tomography (OCT) retinal imaging using the spectral-domain iVue OCT system (OptoVue, Fremont, CA, U.S.A.).

## Eyes processing and immunohistochemical analysis of the retina

Postoperative imaging was performed on days 3, 7, 16 and 30 after surgery under sedation (intramuscular injection of TKX mixture). The pigs were euthanized as per protocol by application of a bolus of 1% propofol (20 mL/animal) followed by exsanguination. After exsanguination the eyes were enucleated and further processed. Whole enucleated eyeballs were fixed in 4% PFA for 24 h at 4 °C, then washed three times with cold PBS. Fixed eyes were embedded in the OCT medium for cryosectioning. Cryosectioned samples were used for immunohistochemical staining. Before processing the enucleated eye, the anterior part was removed, and the eyeball was cut in the sagittal plane through the optic nerve to separate the inferior and superior parts with the corresponding injection sites; 30 µm-thick sections were obtained using a Leica CM3050S cryostat. Cryosections were dried for 4 h at room temperature. Cryosections were blocked in 20% normal goat serum, 0.5% Triton X-100 PBS solution for 1 h at room temperature. Then, an incubation with primary antibodies (anti-turboGFP [Evrogen #AB513] and anti-RPE65 [Abcam #ab235950] was performed overnight at 4 °C. Then, cryosections were washed in 0.5% Triton X-PBS and incubated with Alexa Fluor secondary antibodies for 2 h at room temperature. Nuclei were counterstained with DAPI. After incubation, cryosections were washed and mounted on slides with Prolong Diamond Antifade Mountant (Invitrogen #P36961). Finally, immunostained sections were imaged using a Zeiss LSM 710 Confocal Laser Scanning Microscope and processed on ImageJ Software.

# Results

Six eyes of three minipigs were operated on in total – randomly assigned to either control or experimental group. In the control group, two eyes were operated using a classical approach (lens-sparing vitrectomy and posterior hyaloid detachment before subretinal injection). In comparison, the other four eyes were injected directly with subretinal injection without vitrectomy surgery (Table 1). Table 2 shows a comparison between the two surgical techniques in both groups. No intraoperative adverse events, such as vitreous hemorrhage, retinal tear, hemorrhage or retinal detachment were observed in any of the animals. Optic nerve was well perfused immediately after injection following both approaches. The average surgical time in the conventional vitrectomy group was in the range of 15-30 min but only 10-15 min in the direct subretinal vitrectomy-sparing group.

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table, please view the 'PDF' tab.

**Table 1. Randomization of animal surgery and euthanasia timeline.**

| Animal number | Right Eye | Left Eye | Time of euthanasia (days) |
|---|---|---|---|
| 1 (B699) | No Vitrectomy | Vitrectomy | 3 |
| 2 (B698) | Vitrectomy | No Vitrectomy | 16 |
| 3 (B688) | No Vitrectomy | No Vitrectomy | 30 |

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**Table 2. Comparison of subretinal vector delivery with and without pars plana vitrectomy.**

| Parameter | Vitrectomy | No vitrectomy |
|---|---|---|
| Number of trocars | 3 | 3 |
| Use of triamcinolone acetonide | Required | Not required |
| Removal of posterior hyaloid | Required | Not required |
| Bleb area | Unaffected | Unaffected |
| Reflux of subretinal drug | Common | Less common if the vector < viscous than the vitreous |
| Vitreous replacement | Balanced solution or tamponade | Preserves natural milieu |
| Changes in oxygenation or inflammatory cytokine release | Yes | No |
| Sclerotomy suturing | Preferable | No |
| Surgical time | Standard | Significantly reduced |

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## Post-operative follow-up

The animals were healthy with no adverse events up to the last follow-up at day 30 post-surgery. Immediately, post-operatively, all the operated eyes had mild conjunctival hyperemia and chemosis which resolved in both the experimental and control groups. There was no purulent discharge and intraocular pressures, measured by tonometry, were normal at all timepoints. Slit-lamp examination showed no intraocular inflammation. Dilated examination on post-op day 3 revealed the presence of residual subretinal bleb in both groups, as confirmed by OCT (Figure 3). This later disappeared by day 7 and the whole retina remained flat in all animals till day 30. All retinotomy holes were and stayed healed. On day 16, the retinae were flat with a mildly visible margin corresponding to the bleb site (Figure 4). At the last follow-up, the animals behaved well with preserved visual function, no cataract formation and all retinae were flat with some depigmentation corresponding to the bleb area (Figure 5).

Figure 3. Post-operative day 3. (A) A color fundus photo of the injected eye shows an area of the bleb site (black arrow). (B) Post-operative optical coherence tomography (OCT) serial retinal scans. The upper half shows a detailed view of the residual post-surgical subretinal bleb (white arrow).

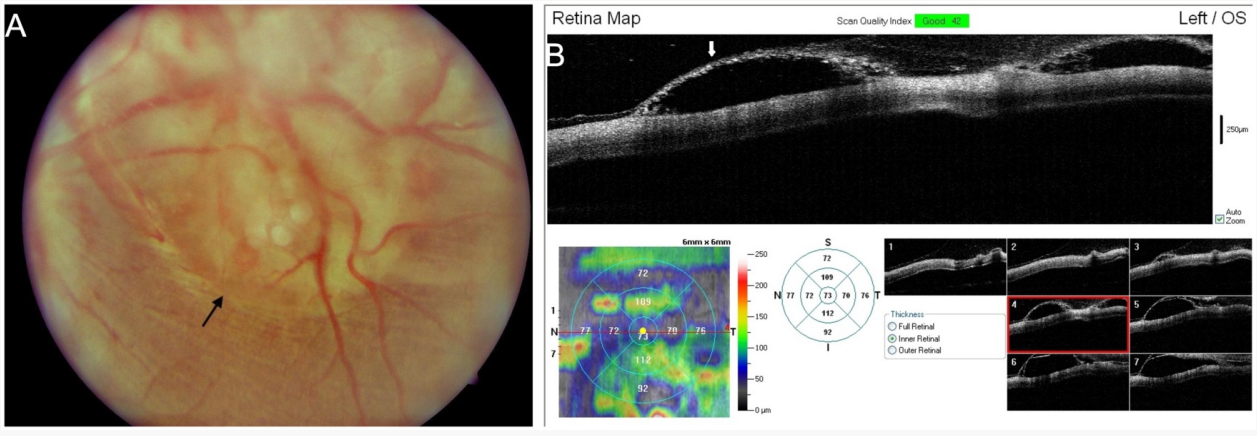


Figure 4. Post-operative day 16. A color fundus photo of the injected eye shows a margin of the previous subretinal bleb (white arrow).

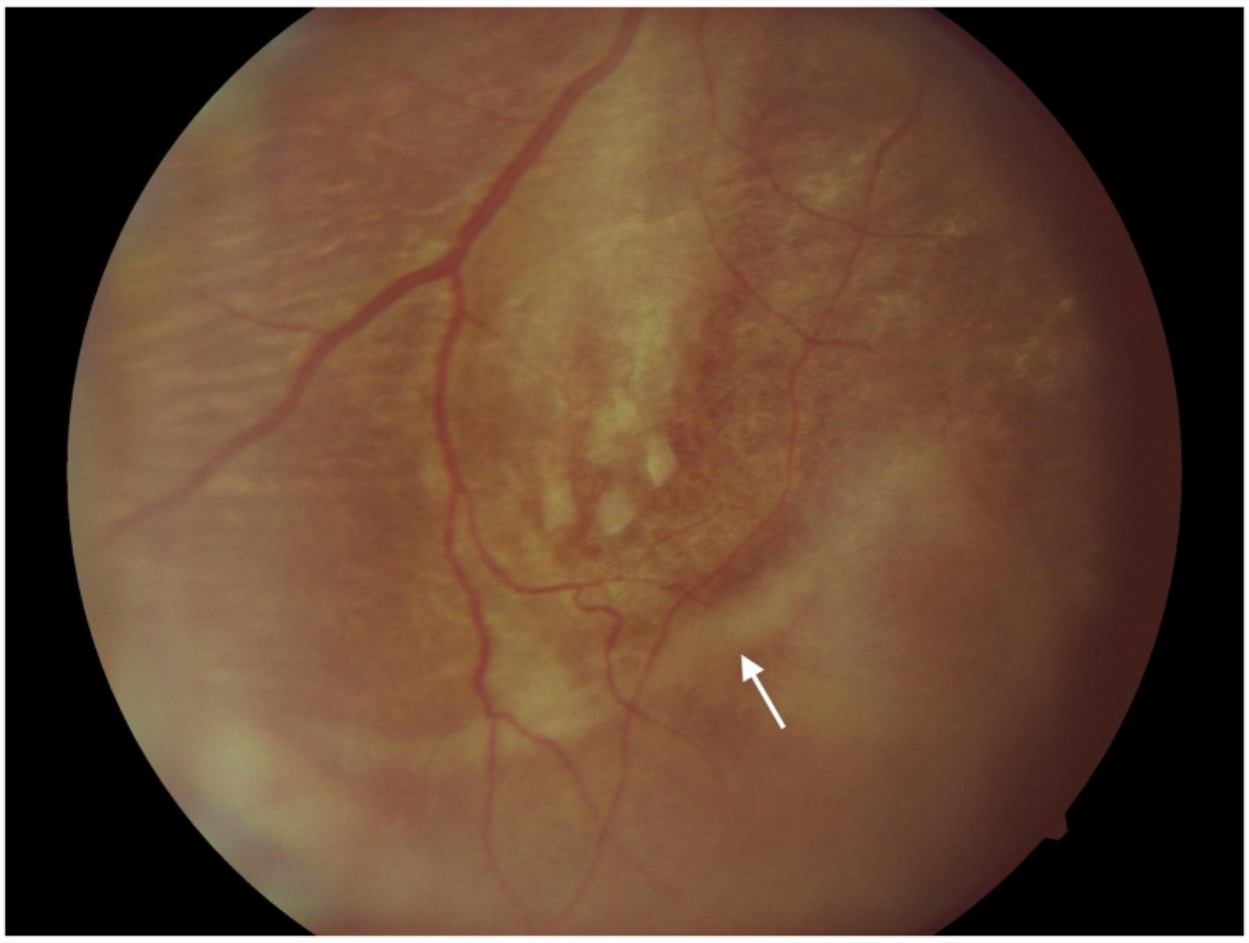
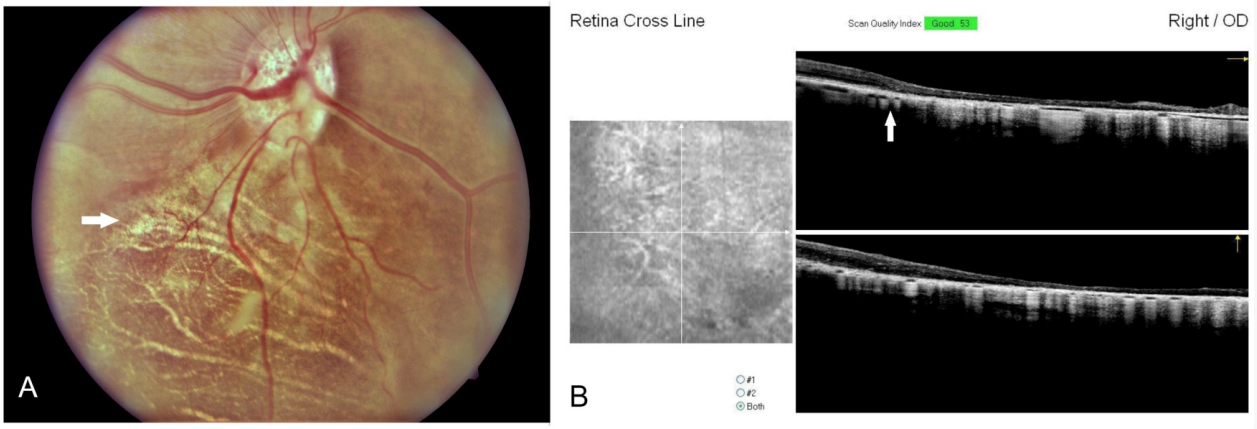


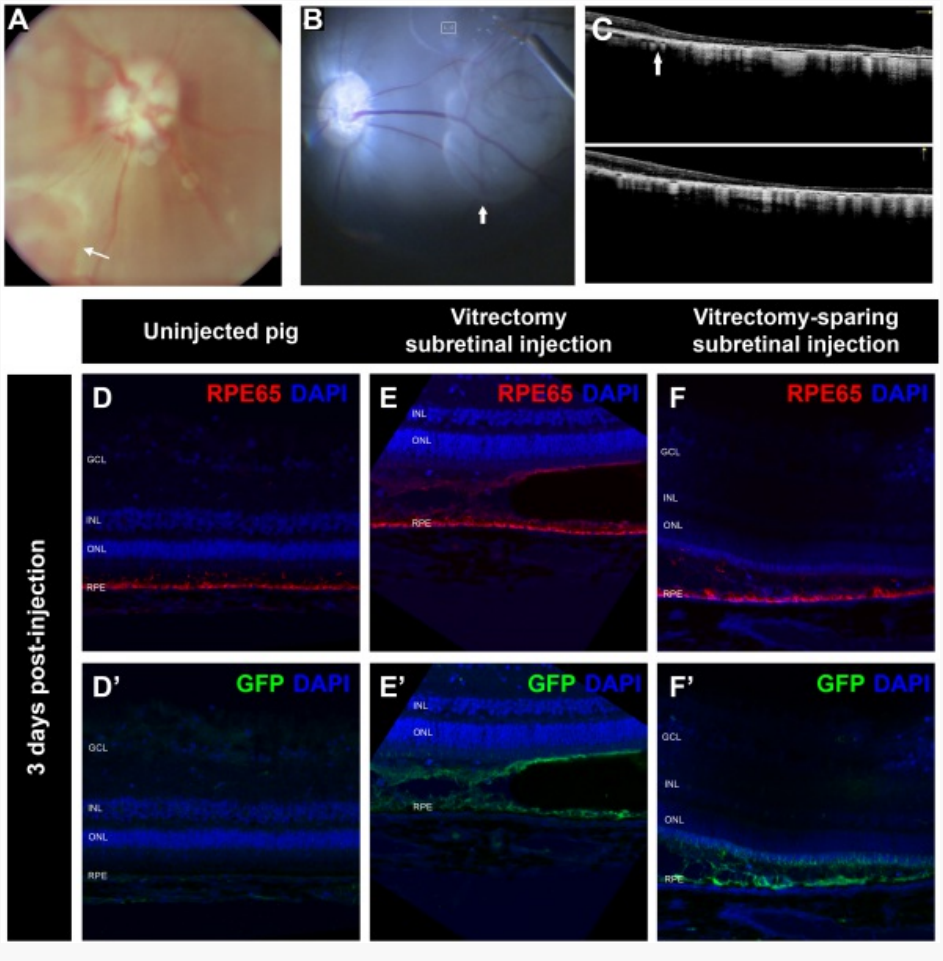
Figure 5. Postoperative day 30. (A) A color fundus photo of the injected eye shows a margin of the previous subretinal bleb (white arrow). (B) Post-operative optical coherence tomography horizontal and vertical B-scans showing attached retina with no bleb and an area of attenuated retinal pigment epithelium and retinal layers (white arrow).




## Histology

Cross sections from the euthanized animal on post-op day 3 were stained for DAPI, RPE65 protein, a protein specifically expressed in the RPE cells, and GFP, to track pS/MAR-CMV-copGFP DNA plasmid expressing cells. As showed by the nuclei staining, both uninjected and injected eye retinal cryosections kept their layered organization with a ganglion cell layer (GCL), inner and outer nuclear layer (INL and ONL). The vitrectomy-assisted subretinal injection displayed a large bleb at the site of injection, with the photoreceptor layer detached from the RPE, while the vitrectomy-sparing subretinal injection maintained the integrity of the photoreceptor cell and RPE layer. GFP expression was found in few photoreceptor and RPE cells at the site of injection, for both vitrectomy and vitrectomy-sparing eyes, hence pS/MAR-CMV-copGFP plasmids efficiently drove transgene expression (see Figure 6).

Figure 6. (A) A fundus photo of an injected eye at day 3 with corresponding intraoperative imaging (B) and one-week post-operative optical coherence tomography imaging (C). Retinal cryosections from an uninjected pig (D-D'), vitrectomy subretinal injected pig (E-E') and vitrectomy-sparing subretinal injected pig (F-F') at 3 days post-injection were immunostained using antibodies for RPE65 in red (D-E-F) and green fluorescein protein (GFP) in green (D'-E'-F'). Cell nuclei were counterstained with DAPI (4',6-diamidino-2-phenylindole) in blue. RPE: retinal pigmented epithelium; ONL: outer nuclear layer; INL: inner nuclear layer; GCL : ganglion cell layer.




## Discussion

This paper describes the technique and initial feasibility of a direct subretinal gene delivery approach with bleb formation but without removal of vitreous in a large animal model. Prior reports describing vitrectomy-sparing direct subretinal injection in gene therapy in bigger animals than mice and rabbits refer to dog models.[28,29] This

report describes an even larger animal model, in comparison to dogs. Pig eyes are bigger in all dimensions and closely resemble the anatomy of human eyes. They also share many similarities with human eyes, having a holangiotic retinal vasculature, no tapetum, cone photoreceptors in the outer retina, and a similar scleral thickness, rendering them valuable in comparative research.[30,31] The pig possesses a relatively large brain with a blood supply and immunologic response similar to those in humans and has been used as an animal model for ocular research, predominantly for late pre-clinical therapeutic development phases.[32,33] Even though the retina of the minipigs does not possess a human-like macula and fovea, it contains the area centralis and visual streaks, which are regions of the retina with a high concentration of cone photoreceptors.[34] This allows histology analysis comparable to human retinal structure. As such, we believe that the same surgical approach in human eyes would yield the same anatomical results and believe that this approach could be feasible and safe in human clinical trials in the near future.

Currently, all human trials investigating or licensing gene therapies have used vitrectomy surgery for bleb formation and subretinal delivery of the product.[5,7] Some surgeons use bleb formation with a balanced salt solution before injection of the vector while some proceed to bleb formation with the solution of the vector itself. An ongoing randomized trial is comparing the efficacy of both approaches.[35] In lieu of the complexity of the whole procedure, the necessity of performing vitrectomy surgery in all cases has been put to question, even though this goes against the standard contemporary paradigms associated with vitreoretinal techniques.[21]

In all our animals the procedures were uneventful and there were no post-operative surgical complications in either of the groups. The experimental procedure without vitrectomy was much faster and less invasive than the control procedure lasting approximately half the time of the control procedure. This was also reflected in faster post-op recovery time in the experimental group perhaps due to induction of less inflammation by surgical procedure. We believe that the vitrectomy-sparing technique has some advantages in comparison to the classical transvitreal subretinal delivery. Apart from shortened surgical time with faster recovery, this approach preserves natural intraocular milieu with intact vitreous and causes less inflammation. Classically, the prerequisite of uneventful subretinal bleb creation was believed to be complete removal of the posterior vitreous including the posterior hyaloid membrane. This belief may not be entirely accurate as we have not observed any difficulty creating the bleb under intact vitreous with attached posterior hyaloid membrane. We acknowledge, however, that more surgeries need to be performed to validate this observation.

Subretinal injections using a transvitreal approach are widely used in clinical studies with viral vectors and cell suspensions through a 38–41 G needle. We have noticed that approaching with such a needle toward the retina, across the vitreous cavity in non-vitrectomized eyes, can be done without any difficulties such as needle bending. The accepted practice for determining the volume of a subretinal bleb is by injection of a predetermined volume of the therapeutic product from a calibrated syringe into the subretinal space. However, based on volumetric estimation using the spherical cap formula of the detached area, 50% or less of the delivered volume reaches the target location.[36] Direct volumetric measurements using intraoperative OCT have shown that subretinal bleb size is on average 36% smaller than predicted by the surgeon using a dilute

triamcinolone solution.[37] In an experimental set-up for cell delivery to the subretinal space, 100% of cases had some degree of reflux.[38] In three of our animals we noticed a minor leak of the injection solution before (hence no reflux) the needle and stream were able to create retinotomy and enter the subretinal space (captured on video). This could be the result of viscosity of our vector. Following the retinotomy creation, we did not seen [please correct to "see"] any reflux of the injected volume. We can speculate that the intact vitreous can be a barrier to reflux in comparison to the vitrectomized intravitreal space into which the vector can escape more easily. This can be accentuated even more in children whose vitreous is more compact and more adherent to the retina than in adults. However, as this is a feature of vitreoretinal interface, the access to subretinal space and ease of injection not [please change to: "may not be..."] be different than in adults. More future gene therapies will be applicable to pediatric population than before.

Another potential concern with direct subretinal injection could be a sudden increase in intraocular pressure in eyes without vitrectomy, especially with injection of large volumes. At the beginning of surgery the IOP was set at 25 mmHg. In eyes with attached infusion during vitrectomy surgery, the eye pressure is automatically regulated and adjusted in case of sudden IOP increase.While this regulatory mechanism is absent during direct subretinal delivery, in our eyes the optic nerves were well-perfused immediately following the subretinal injection of 0.1 ml volume. Whether higher injection volumes are still tolerable or results in an increase in IOP as with intravitreal injection, remains to be investigated.

Our study utilized a DNA plasmid vector as a prototype for assessing surgical technique. Recently, these S/MAR DNA vectors have successfully packaged large genes, such as USH2A, with persistent transgene expression in the zebrafish retina for up to 12 months post-delivery.[14] Non-viral gene delivery systems have several characteristics that make them attractive as vehicles including low immunogenicity, safety, and unlimited size capacity (AAV vector can only hold genes <4.7 kb), however, they have a lower transduction efficiency, which can limit their therapeutic effectiveness.[4] This study reports on the first use of non-viral vectors in a large animal model. As demonstrated by immunohistology, the vector used in this study showed high expression levels in the RPE and inner retinal cells. The retinal bleb areas have shown a mild depigmentation at the last post-op follow-up and this was likely due the Jet-PEI, which is known to be retinotoxic. Further work is required to develop efficient nanoparticles that can aid the transfection capability of non-viral genetic therapies including DNA and mRNA. While the focus of this study was on a new way of subretinal drug delivery rather than on efficacy of non-viral gene delivery, this surgical approach would be beneficial especially for non-viral gene delivery.

In summary, this experimental study demonstrates the technical feasibility and initial efficacy of vitrectomy-sparing direct subretinal non-viral vector gene delivery in the largest animal model reported. In comparison to the transvitreal subretinal approach using vitrectomy surgery, our approach highlights numerous advantages including shorter operating time and recovery while reaching the same goal. We hope that these experiments might serve as evidence and encouragement to apply this approach to human subretinal gene delivery studies and trials. Before that, however, we acknowledge the need to enlarge the study and do more experiments in the

future to validate the results.

## Acknowledgement

We wish to acknowledge contribution of Drs. Jana Juhásová a Štefan Juhás for providing anesthesia to animals in the experiment.

## Ethical approval

All experiments were carried out according to the Guidelines for the Care and Use of Laboratory Animals and according to the Association for Research in Vision and Ophthalmology (ARVO) for the use of animals in ophthalmic and visual research. The study protocol was approved by the Resort Professional Commission of the CAS for Approval of Projects of Experiments on Animals at the Institute of Animal Physiology and Genetics of theCzech Academy of Sciences (Liběchov, Czech Republic) (Approved protocol No. 64/2022). During manuscript preparation we have followed The ARRIVE Guidelines 2.0: updated guidelines for reporting animal research of the EQUATOR Network.

## Authors' contributions

Conception and design (TA, ZE, JM, MM, IK), Methodology (TA, YN, ZE, JM, MM, IK), Data acquisition (ZS, YN, MT, LT, RH, MM, IK), Data Analysis (ZS, MT, LT, ZE, JM, MM, IK), Manuscript writing (ZS, YN, MT, RH, ZE, LL, GP, JM, MM, IK), Manuscript Review (ZS, TA, LL, GP, MM, IK).

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Data availability statement

Data is contained within the article and supplementary material.

**Note:** this Edit/html view does not display references as per your journal style. There is no need to correct this. The content is correct and it will be converted to your journal style in the published version.

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