Video Article

A Step by Step Protocol for Subretinal Surgery in Rabbits

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Abstract

Age related macular degeneration (AMD), retinitis pigmentosa, and other RPE related diseases are the most common causes for irreversible loss of vision in adults in industrially developed countries. RPE transplantation appears to be a promising therapy, as it may replace dysfunctional RPE, restore its function, and thereby vision.

Here we describe a method for transplanting a cultured RPE monolayer on a scaffold into the subretinal space (SRS) of rabbits. After vitrectomy xenotransplants were delivered into the SRS using a custom made shooter consisting of a 20-gauge metallic nozzle with a polytetrafluoroethylene (PTFE) coated plunger. The current technique evolved in over 150 rabbit surgeries over 6 years. Post-operative follow-up can be obtained using non-invasive and repetitive in vivo imaging such as spectral domain optical coherence tomography (SD-OCT) followed by perfusion-fixed histology.

The method has well-defined steps for easy learning and high success rate. Rabbits are considered a large eye animal model useful in preclinical studies for clinical translation. In this context rabbits are a cost-efficient and perhaps convenient alternative to other large eye animal models.

Video Link

The video component of this article can be found at https://www.jove.com/video/53927/

Introduction

Age-related macular degeneration (AMD) is the most common cause of visual impairment in adults aged 50 or older in industrially developed countries, as it causes loss of central vision. About 15% of these patients suffer from the "wet" form of the disease, in which neovascularization originates from the choroid and disrupts retinal function1. This variant can be treated by a highly effective therapy with repeated intra-vitreal injections of antiangiogenic drugs2. However, the vast majority of patients (~85%) suffer from the dry form, which is characterized by extracellular deposits (e.g., drusen) under the retinal pigment epithelium (RPE). These deposits cause RPE dysfunction leading to retinal atrophy in the macula. Given the lack of any curative therapeutic options, AMD evolved into an intensively developing research field, where many different curative therapeutic approaches are being tested. Surgical RPE replacement is one attractive future possibility to defeat this debilitating disease.

Autologous subretinal RPE transplantation replaces dysfunctional or lost RPE in macula, and has the potential to restore its physiological function3-9. This surgical technique had a breakthrough with the development of RPE differentiation protocols from human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC), giving the scientist an unlimited cell source of RPE for transplantation10. RPE transplantation is now recognized as an attractive first-in-human application for stem cell derived therapeutics. The eye offers excellent surgical access and sophisticated in vivo monitoring tools11-13.

To transplant the RPE, one way is with a minimally invasive delivery using a cell suspension, alternatively, to better preserve RPE characteristics and transplant function, artificial carrier substrates (scaffolds) for RPE replacement are being considered14,15. Large animal models are required for preclinical validation, yet detailed technical information on animal handling and surgical technique is missing to date16-23.
We and others\textsuperscript{11,24} despite some evidence to the contrary\textsuperscript{25}, suggest the use of a rigid yet elastic carrier substrate as it provides safer handling, preserves monolayer integrity and functionality. Over time we have tested several custom-designed instruments and ancillary techniques for the implantation of cell-carrier supported RPE transplants into the subretinal space (SRS). We utilized intraoperative video recordings, in vivo scanning laser ophthalmoscopy combined with spectral domain optical coherence tomography (SLO/SD-OCT), and histology to evaluate the implantation success\textsuperscript{14,26,27}. Here we provide our current recommendation for subretinal RPE implants in rabbits, which were tested in 5 different rabbit strains, 7 cell carrier materials and 4 RPE cell sources in over 150 procedures.

### Protocol

**Ethics of animal handling in ophthalmic research:** We obtained approval from the Ethics committee of the Medical Faculty, University of Bonn, and adhere to the guidelines stated by The Association for Research in Vision and Ophthalmology (ARVO). Moreover, all procedures were approved by the state regulatory authorities of North Rhine-Westphalia. Animals were held indoors in a specialized facility in an air-conditioned room with temperatures between 18 - 20 °C, exposure to regular daylight, in standardized individual cages with free access to food and water.

Note: To ensure the animals operative affinity, an animal health score sheet is followed which includes the following definitive animal exclusion criteria: 20% weight loss compared to weight on admission; apparent cyanosis of the animal; animal shivers, has cramps or cannot move in coordination; ataxia/paresthesia, e.g., paralyses; apathy; extreme auto mutilation (skin wounds, severed limbs).

1. **Instrument Sterilization**
   1. Place reusable instruments in ultrasonic bath.
   2. Add 500 ml distilled water and 2 ml of instrument disinfectant.
   3. Clean instruments using sweep function for 15 min.
   4. Remove instruments from ultrasonic bath and rinse thoroughly using distilled water for 5 min.
   5. Insert instruments in autoclave and use the standard program (sterilization of instruments under 121 °C for 20 min).

2. **Instrument Preparation**
   1. Establish and maintain a sterile field, by working in a closed room, wearing surgical scrubs, mask and hair cover. Disinfect hands prior to wearing sterile surgical gloves. For detailed approach see\textsuperscript{28}.
   2. Place sterilized instruments on a sterile drape.
   3. Place 1 ml syringe filled with 40 mg triamcinolone attached to a 27 G needle for injection, 10 ml syringe with Balance salt solution (BSS), and 5 ml syringe of lubricant on drape.
   4. Place 3-0 silk, 7-0 vicryl, ocular sticks (to stop conjunctival/ scleral bleeding), twister gauze sponges, wound closure strips (to fixate the vitrectomy tip tubing), and chandelier endoillumination fiber wire on a drape.
   5. Unwrap 25 G chandelier endoilluminator and connect to light machine using sterile techniques (see step 2.1). Connect vitrectomy set including high speed vitrector and Venturi cassette to vitrectomy machine using sterile techniques (see step 2.1).
   6. Open 500 ml BSS bottle and connect solution to Venturi cassette according manufacturer's instructions.

3. **Preparation of Anesthesia and Positioning of the Animal**
   1. Weigh animal to ensure accurate medication dosage.
   2. Prepare intramuscular (IM) anesthesia using 1 syringe with 27 G needle containing 0.35 mg/kg ketamine and 0.25 mg/kg medetomidine for start. Flip the syringe to mix.
   3. Prepare 2 syringes with 1/3 the doses to maintain the anesthesia during the operation.
   4. Prepare syringe containing 20 ml of 5% glucose solution and 18 G needle for subcutaneous injection as an intravenous infusion alternative.
   5. Give 3 x 1 drop of mydriatic eye drops prior to vitrectomy for pupil dilatation.
   6. Cover rabbit with blanket to calm before anesthesia injection, inject in the hind limb (gluteal muscle) and massage around injection site for 30 sec.

   Note: The first shot of IM anesthesia lasts about 1 - 2 hr. depending on the rabbit's size, drug tolerance, fat layer, stress and body temperature. The first sign of the anesthesia fading away is a nystagmus (must be monitored by the surgeon), subsequent injections last about 30 - 45 min.

   7. Confirm proper anesthesia, by verifying hypnosis, hyporeflexia, analgesia and muscle relaxation of the animal.
   8. Give subcutaneous injection (Pt. 3.3) in neck skin fold, once the rabbit is unconscious.
   9. Add methylcellulose lubricant every 5 - 10 min in operated eye, add lubricant and tape lids in non-operated eye.
   10. Place the covered rabbit on the surgical table draped with a cover such as a cotton blanket in an optimal position (nose slightly elevated through a mold of the blanket, so it is level with ocular surface) under surgical microscope. Align eye perpendicular to microscope objective.
   11. Ensure proper body core temperature using rectal thermometer (normothermia 39 ± 1 °C)\textsuperscript{29}.
   12. Cut eyelashes using scissors (some ointment on blade) to reduce postoperative infections.
   13. Disinfect the eye using 2 - 3 drops of 0.1 g/ml Povidone-Iodine topically for 1 min and rinse with sterile BSS.
   14. Cover eye with sterile drape with pre-cut opening in the middle for the eye and then cover with (sticky) surgical incision drape 12 x 17 cm.

4. **Vitrectomy**
   1. Proptose and secure eye with 3-0 silk using inverted caliper and, perform a conjunctival peritomy.
1. Incise the conjunctiva with a vannas scissor close to limbus but far enough from the blood vessels (~1 mm distance).
2. Dissect the conjunctiva by creating a “T-cut”. First enlarge the peritomy with the scissor parallel to limbus and then incise the conjunctiva vertically in form of a “T” for about 6 - 7 mm. Carefully separate the conjunctiva bluntly.

2. Perform a sclerotomy using a 23 G microvitreoretinal (MVR) blade at 8 o’clock on right eye/OD (4 o’clock on left eye/OS) by carefully inserting the sharp tip of the blade in direction towards the optic nerve. Slowly retract the blade in the same direction and avoid enlarging the sclerotomy.
3. Insert and suture custom side port-infusion cannula\textsuperscript{27} using 7-0 silk suture and set intraocular pressure (IOP) at 24 mmHg.
4. Perform a sclerotomy with 25 G flat head trocar at 2 o’clock on OD (10 o’clock on OS) similar to step 4.2.
5. Insert 25 G chandelier light into flat head trocar, fixate with sticky tape and turn on light source at ca. 30%.
6. If needed, remove edematous corneal epithelium using a #20 scalpel for better intraocular visualization.
7. Perform a sclerotomy similar to step 4.2 at 10 o’clock on OD (2 o’clock on OS), (pre-) place u-shaped 7-0 sutures around sclerotomy without tying the knot, and insert vitrectomy cutter tip.
8. Start vitrectomy\textsuperscript{30} around the entry port, then continue over the optic disc and the fibrae medullares using high speed vitrector by cutting the vitreous humor into small pieces at max. 2,000 - 3,000 cuts/min, aspirating at max. 200 mmHg using the stated parameter setup of the vitrectomy machine (Table 1).
9. Perform a posterior vitreous detachment (PVD) by separating the vitreous humor from the retina by holding the high speed vitrector over the posterior pole and (if feasible gently) superior of the disc\textsuperscript{31} while aspirating only at max. 200 mmHg without cutting.
10. Inject ca. 50 µl (20 mg) triamcinolone or diluted fluorescein (ca. 0.1 mg/ml) intravitreally to visualize and facilitate (near total) removal of the floating vitreous over the posterior pole and midperiphery during vitrectomy. Avoid crossing over under the lens. Indentation to shave peripheral vitreous by a (skilled) assistant is recommended if gas tamponade is desired.
11. Add 20 units/ml heparin and 0.5 mg epinephrine to final concentration of 0.001 mg/ml into the BSS infusion solution in parallel or after step 4.10.

Note: As heparin/epinephrine are not injected intraocular their effects are delayed depending on infusion flow rate.

5. Loading Shooter

Note: The work described herein does not fall under the tenets of the Declaration of Helsinki; it did not involve human patients. Here, standard RPE cells were isolated from fetal human eyes, cultured and differentiated on uncoated 10-µm-thick polyester (PET) inserts according to our previously published protocol\textsuperscript{14}. A permission to work with the human fetal material was obtained from the ethics committee of the University of Bonn. Alternatively, HES-RPE were shipped from the Skottman lab (manuscript in prep.), where they were cultured according to the technique described by Vajajasaari et al.\textsuperscript{22}; for these cells a permission has been obtained from the R. Koch Institute, Berlin, Germany.

1. Rinse cell culture prior to preparation of the implant 3x with ophthalmic grade BSS.
2. Fill a standard cell culture dish (100 x 20 mm) with 10 ml ophthalmic grade BSS.
3. Add the cell culture insert into the BSS and center the dish under a light microscope.
4. Punch out a 2.4 x 1.1 mm implant with a blunt, oval, custom-made needle to obtain a flat, bean-shaped substrate with two long edges and two round edges.
5. Gently flood the needle through the second port with BSS to flush out the implant into the BSS filled custom made loading station (Figure 1).
6. Optionally cut one round end of implant (<0.5 mm), just to obtain a third edge.
7. Make sure that the implant is in the right orientation by ensuring that the monolayer is upside on the cell carrier. To change positioning carefully use two scalpels.
8. Push the implant gently and completely into the shooter instrument using the needle holder until all of the implant is secured inside of the tip.
9. Keep the “loaded” shooter tip in loading station under BSS until the moment of implantation.

6. Implantation

1. Approach neural retina with extendible 41 G subretinal injection needle connected to a gastight syringe (ensure that all air bubbles have been evacuated from tubing!).
2. Inject BSS (with calcium and magnesium/ CM) subretinally and thereby create a bleb retinal detachment (bRD) of about 2 - 3 disc diameter (DD). Two BRD per eye can be raised safely.
3. Enlarge retinotomy to 1.5 mm with vertical 23 G VR-scissors. The subretinal space is now accessible for implantation or further maneuvering.
4. Extend sclerotomy (precisely) with a 1.4 mm incision knife to 20 G approach.
5. Attempt passing through the sclerotomy using a 20 G shooter dummy, enlarge as needed to ensure smooth, yet snug transition of the loaded shooter.
6. Pass with the loaded shooter\textsuperscript{27} through sclerotomy ideally at 24 mmHg.
7. Approach retinotomy edge and eject the implant subretinally from an epiretinal position.
8. Adjust the implant with half-closed 23 G scissors, forceps or 41 G needle to make sure it is positioned well under the retina- reasonably away from the retinotomy.

7. Ending Operation

1. Remove 25 G chandelier and infusion cannula.
2. Suture all sclerotomies.
3. Inject 25 µl (10 mg) triamcinolone by the 8 o’clock sclerotomy (prior to suturing last sclerotomy).
4. Check/adjust IOP by palpation and inject BSS via 30 G needle/syringe, if needed.
5. Suture conjunctiva with 7-0 vicryl.
6. Remove propitosing 3-0 silk sling slowly (Avoid deep orbital venous plexus!).
7. Add dexamethasone/antibiotic ointment under lid.
8. Position for 1 hr covered with blanket with operated eye facing up (w/o gas), or down (with air/gas).
9. Do not leave animal unattended until it regains sufficient consciousness to maintain sternal recumbence.
10. Do not transport rabbit before the anesthesia fades completely, this can be speeded up by injecting medetomidine reversing agent equal to amount of medetomidine given.

8. Post-operative Animal Care

1. Keep rabbits under appropriate conditions (temperature, light, food, water, space, etc.) and close monitoring in a specialized facility.
2. Ensure that animal is well rested, i.e., no extended periods of food or water deprivation.
3. Look for any wounds or injuries, especially on injection sites.
4. Keep wounds dry to prevent infections. Give antibiotics when infection is suspected: dexamethasone 1 mg/g, neomycin sulfate 3,500 I.U./g, polymyxin B sulfate 6,000 I.U./g ointment was applied twice daily for 1 week postoperative onto the ocular surface.
5. Add dexamethasone/antibiotic ointment for next 7 days postoperative twice daily for better ocular surface regeneration and reduced postoperative pain.
6. Do not leave an animal unattended until it has regained sufficient consciousness to maintain sternal recumbence.
7. Do not return an animal that has undergone surgery to the company of other animals until fully recovered.
8. Do not expose animals to unnecessary distress.

9. SLO/SD-OCT Guidance

1. Prepare and inject intramuscular (IM) anesthesia using 1 syringe with 27 G needle containing 0.175 mg/kg ketamine and 0.125 mg/kg medetomidine for start. Flip the syringe to mix.
2. Add a lubricant at least every 5 min to moisturize eye and maintain clear SD-OCT imaging, optionally a custom contact lens may be used.
3. Attach a steel platform to the headrest to stabilize the animal in the required position.
4. Place rabbit on steel platform, placing its eye perpendicular to probe.
5. Hold the rabbit's head from the lower cheek bone (mandibula) avoiding the trachea.
6. Tilt animal's head by roughly 45° towards the SD-OCT probe to optimize the viewing angle on the implant.
7. Use a 30-degree lens and the following parameters for optimal OCT imaging [HS]: 30 degrees settings for single line scans with ART mode set to 100 (averaging) and 20 x 20 degree settings for volume scans with ART mode set to 15; high resolution mode is not required.
8. Use SLO infrared reflectance imaging to find the focal plane of the implant (Fig. 2A); optimal focus is reached when (all) the implant edges are sharp. 
9. Add dexamethasone 1 mg/g, neomycin sulfate 3,500 I.U./g, polymyxin B sulfate 6,000 I.U./g ointment under lid when finished.

Representative Results

The results from the described method for subretinal implantation are shown in Table 2. Engraftment under the retina had a success rate of ca. 61% when a core vitrectomy was performed and rose up to 76%, when posterior vitreous detachment was induced. These numbers include ca. 21% of animals who died either intraoperatively or in the first 3 postoperative days. This technique can be used to implant two scaffolds on different retinal areas in one eye simultaneously.

The rabbits underwent postoperative in vivo follow up using SD-OCT and histologic processing as described by Stanzel et al.27 (Figure 2).

Fig. 2A shows scanning laser ophthalmoscope infrared reflectance image of implanted cultured RPE on a polyester membrane (PET) after an uncomplicated transplantation. The halo around implant corresponds to photoreceptor atrophy. Fig. 2B shows corresponding SD-OCT, notice retinal, mainly outer nuclear layer (ONL) thinning, hyper reflective band on SD-OCT above implant, while the neural retina adjacent to the implant shows near-normal reflection bands. These results suggest an atraumatic delivery. Fig. 2C shows Hematoxiline/Eosin (H/E) stain of the implant which shows subretinal scarring and ONL atrophy around the retinotomy site likely as a result of iatrogenic manipulation, a contiguous yet irregular pigmented layer over PET. Bruch's membrane underneath the implant also appeared to be contiguous, and the choriocapillaris contains some scattered erythrocytes. These morphologic result are comparable to the SD-OCT and strengthen the thesis of an atraumatic delivery.
Figure 1: Loading Shooter with Human iPSC-RPE Cultured on PET Cell Carrier. A) Shows punching out an implant using custom made needle. B) Bean-shaped implant cut from cell culture. C) Positioning of implant before loading. D) Loading of shooter with implant. Please click here to view a larger version of this figure.

Figure 2: Cultured Human hES-RPE on PET Cell Carrier 4 Weeks in Rabbit Subretinal Space. A) Shows SLO infrared reflectance image, the green line demarcates the cross-section shown in Fig. 2B. B) Corresponding SD-OCT. C) H/E stain, see text or 26,27 for details. Please click here to view a larger version of this figure.

<table>
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<tr>
<th>Parameter</th>
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<td>Vitrectomy</td>
<td>6,000 cuts/min</td>
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<tr>
<td>Vacuum</td>
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<td>Rise time</td>
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Table 1: Parameter Setup of the Vitrectomy Machine.
We caution however, as several intra-operative complications may occur any time, hindering the success of the implantation, for instance intraocular bleedings, anesthesia fading off during vital steps such as the implantation, collapsing of bRD due to instrument manipulation or ocular hypotony, rabbit death due to excessive doses of anesthesia, low blood pressure during long operation causing hypoxic brain damage, or hyperthermia. Yet these complications decrease with time as they are quickly tackled and resolved by increasing experience of the surgical team.

Some complications could be reduced by following a few simple, yet crucial steps. Lubricant should be added every 5 - 10 min to prevent corneal, scleral and conjunctival damage during the operation, and to maintain a clear intraocular media, as dried/blackened sclera may be a cause of wound dehiscence, which in turn leads to ocular hypotony and/ or intraoperative leakage from sclerotomies. Heparin should be added to prevent the formation of a fibrin film that makes particularly subretinal implantation challenging and simultaneously adding epinephrine to reduce bleeding under heparin. Too long heparin/ epinephrine exposure times (>1 hour) should be avoided to prevent corneal edema by endothelial decompensation, hypertensive crisis or intraoperative fatality. Meticulous vitreous removal should be performed at instrument (entry) port to avoid retinal and/ or choroidal detachments. Intraocular instruments should be pointed towards posterior pole to avoid lens touch (causes iatrogenic cataract formation) or (entry site) retinal damage. An intracocular side-port infusion cannula should be used, as it attenuates the jet stream around the implantation area, thus preventing uncontrolled tearing of the retinotomy, and collapse of the bRD. In the midline (vertical axis from optic nerve) or close to optic medullar fibers should be avoided to prevent extensive iatrogenic retinal detachments. Finally, last but not least bRD should be induced at low IOP, to avoid subretinal BSS injection using excessive flow rates which may lead to a retinal damage (e.g., by stretching).

Many study variables such as cell carrier variants, fetal, adult or stem cell derived RPE cell sources, choices for immunosuppressants, etc., can be explored. Further improvement such as serum-free RPE culture methods, characterization of xenoRPE in subretinal space, removal of the host RPE layer or strategies for implant anchorage are current work in progress.

To date the described techniques have been used on 5 different rabbit strains, including chinchilla bastard, Chinchilla bastard/KBL hybrids, New Zealand White/Red Cross, New Zealand White (albino) and Dutch belted. Both male and female rabbits were operated on, with rabbits at least 1.5 kg or 2 months of age (depending on species). Most surgeries were on pigmented rabbits (chinchilla bastard or chinchilla bastard hybrids) with weights between 2.5 - 3 kg.

All of the rabbit strains we have had the opportunity to work with seem to have some peculiarities. Given the exclusive availability of pigmented rabbits of the chinchilla bastard strain in Germany in 2009-13, we have collected the most experience with these animals. Unfortunately it is no longer available, since breeding has been discontinued, but compares very well to New Zealand White/Red Cross except for the more advantageous thicker sclera and larger eye volumes in the latter. Chinchilla bastard hybrids have significant intraoperative fibrin formation and require heparin/epinephrine use as outlined above to ensure successful subretinal maneuvers. This protocol has also been performed in non-pigmented albino rabbits (New Zealand White), however particularly bRD creation and subretinal implantation is more challenging given reduced contrast appreciation. The feasibility of inducing a posterior vitreous detachment did not seem rabbit strain dependent in our hands.

Transvitreal subretinal delivery is likely the future surgical strategy of choice given it is the most common route nowadays clinically to access the retina. As a result many other groups have presented such techniques for cultured RPE on carrier supports in animal work. Aramant et al. have an instrument, which places rather than pushes their hydrogel-encapsulated soft implant to its subretinal target site. The design of the insertor instrument for the delivery of subretinal chip implants in pigs but no further work has been published since to the best of our knowledge. We have been able to extend the described technique with some modification to pig.

Our preferred cell carriers are 10 micron thick polyester terephthalate (PET) membranes. From a surgical perspective, this material has favorable stiffness and elasticity parameters, in addition to its broad versatility during cell culture experiments. We found similar experiences with expanded tetrafluoroethylene (ePTFE) or nanofiber membranes electrospun from PET, poly-lactic/capronolactic acid (PLCL) or poly-lactic-co-glycolic acid (PLGA), as well as composite nanofiber (PLGA or PET) and ultra thin PET. When PET membranes are used with our metallic shooter
instrument, they do have an occasional tendency to exhibit electrostatic charge, which challenges their ejection from the shooter. Ultra thin polyimide membranes could in our hands not be implanted in the subretinal space with the protocol outlined above (manuscript in preparation).

Marmor et al. have systematically studied spontaneous resorption of subretinal fluid in iatrogenic localized retinal detachments. Even following manipulation in the subretinal space these were found to be reabsorbed by postoperative day 4 in uneventful surgeries. Laser retinotomy is not performed to secure the edges of the retinotomy. Although counterintuitive when compared to human surgery, air/gas tamponade is not required. Unless meticulous removal of peripheral vitreous can be achieved, particularly in the superior quadrant, this may in fact result in giant retinal tears originating from the retinotomy site. It is only recommended to perform fluid air exchange with subsequent 20% SF6 gas tamponade to salvage intraoperative iatrogenic retinal detachments or in case a particular implant position needs to be secured.

Although the mechanically induced ablation of the neural retina can cause RPE and photoreceptor damage in rabbits, its extent varies greatly (even with regular BSS) depending on factors such as IOP, syringe type used, injection volume with thereby induced retinal stretching. We have also tested the often recommended Ca/Mg-free BSS facilitated detachment, but found that it causes intraoperative lens opacification (particularly with elevated temperature), and significantly delays or even impairs retinal re-attachment. Slow subretinal injection of 20-30 µl volume of regular BSS with a 100 µl syringe is therefore recommended; injection needle movements should be minimal so the retinotomy seals around it and prevent Bruch's membrane damage. Some of the iatrogenic damage may be resolved by RPE wound healing, and the observed relative preservation of ONL thickness after reattachment, suggests that the RPE/photoreceptor complex can tolerate this impairment, as also described by others.

Cell-based therapeutics or retinal prosthetics require preclinical animal testing prior to regulatory approval and commencing human safety studies. The former vary from country to country. The rabbit model described here may serve as a cost-efficient and less challenging platform for establishing or even carrying out all requirements by regulatory authorities. Moreover, it may subsequently serve for training of surgeons in eventual multicenter clinical trials or further improvements of the technique along the way.

Disclosures

RB, BVS, ZL, NE and Geuder AG have filed a European patent application on the shooter. NB is an employee of Geuder AG. Publication fees for this video-article were paid by Geuder AG.

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