# Chapter 6
# Surgical Approaches for Cell Therapeutics Delivery to the Retinal Pigment Epithelium and Retina


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**Abstract** Developing successful surgical strategies to deliver cell therapeutics to the back of the eye is an essential pillar to success for stem cell-based applications in blinding retinal diseases. Within this chapter, we have attempted to gather all key considerations during preclinical animal trials.

Guidance is provided for choices on animal models, options for immunosuppression, as well as anesthesia. Subsequently we cover surgical strategies for RPE graft delivery, both as suspension as well as in monolayers in small rodents, rabbits, pigs, and nonhuman primate. A detailed account is given in particular on animal variations in vitrectomy and subretinal surgery, which requires a considerable learning

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curve, when transiting from human to animal. In turn, however, many essential subretinal implantation techniques in large-eyed animals are directly transferrable to human clinical trial protocols.

A dedicated subchapter on photoreceptor replacement provides insights on preparation of suspension as well as sheet grafts, to subsequently outline the basics of subretinal delivery via both the transscleral and transvitreal route. In closing, a future outlook on vision restoration through retinal cell-based therapeutics is presented.

**Keywords** Age-related macular degeneration · Retinal pigment epithelium · Photoreceptor · Transplantation · Cell-based therapy · Cell replacement · Surgery · Anesthesia · Mouse · Rat · Rabbit · Pig · Nonhuman primate · Monkey · Preclinical study · Vitrectomy · Immunosuppression

## 6.1 Introduction

Delivery of novel therapeutic agents to the retina, in particular for cell replacement of the retinal pigment epithelium (RPE) and photoreceptors (PR), has gained considerable interest since the introduction of efficient protocols for the generation of pluripotent stem cell (PSC)-derived cell transplants for clinical application. Initial feasibility of RPE transplantation was demonstrated using primary cell sources by Gouras et al. in monkeys [1] and subsequently also for photoreceptors in rats by Silverman and Hughes [2]. Surgical clinical experience with replacement of dysfunctional or lost RPE in age-related macular degeneration (AMD) or photoreceptors in retinal dystrophies has been available since the 1990s [3, 4].

Since then, diverse distinct animal models as well as treatment modalities in patients have been explored. While the RCS rat remains the gold standard for visual testing for the FDA, athymic rats and humanized mice enable high-throughput testing, such as teratogenicity assays of human PSC-derived retinal cells as regards human immune system characteristics. Rabbits and pigs enable refinement of surgical instrumentation and techniques, as well as immune suppression protocols immediately applicable to human use, with nonhuman primates offering a uniquely precious opportunity to validate all prior findings in a foveate animal.

Given the complexity of photoreceptor transplantation a number of different inherited mouse models characterized by photoreceptor dysfunction and degeneration have been introduced. These include autosomal recessive, dominant, and X-linked models affecting either rods, cones, or both photoreceptor types. The different mouse models of retinal degeneration were derived either by spontaneous mutations or genetic engineering. Phenotypically, the available mouse models often resemble the disease phenotypes observed in humans. Particularly inherited retinal degeneration can be quite precisely modeled by gene-modified mice, including rapid to slow degeneration patterns spanning time lines from a few days to several

months for the loss of main parts of the ONL. However, proper mouse models for more complex retinal diseases like AMD have not been established. While specific aspects of AMD have been recapitulated in rodent models, the complete spectrum could not been reproduced, what might be not surprising given, for example, the short life span of nocturnal rodents besides the lack of a macula. Though mouse retinopathy models have been and further will be of utmost importance for developing photoreceptor transplantation strategies for the treatment of retinal degenerative diseases, the use of large, diurnal animal models is more and more recognized as an important translational step toward clinical application. Indeed, first pig models with inherited retinal dystrophies have been generated and given the recent advancements in gene editing using CRISPR/Cas technology more specific models are expected in the near future. Of interest may be further already available dog strains, that suffer from spontaneous mutations in vision related genes due to intensive inbreeding for the generation of particular strains [5]. Given the existence of a macula in nonhuman primates these animals represent highly reliable animal models. However, besides limitations in regard to organization, finances, time, and regulatory requirements for experimental work with monkeys, there are currently no genetic primate models of inherited retinal degeneration available. Though, first transplantations studies using human embryonic stem cell-derived retinal tissue were performed in photoreceptor degeneration monkey models, generated by acute chemical or light/laser damage [6].

Clinically, RPE delivery under the macula has been achieved with cell suspensions, RPE-choroid patches, isolated RPE sheets—both cultured and uncultured—and recently aided by a cell carrier as a cultured monolayer from human embryonic PSCs. Clinical subretinal neural retina transplantation techniques have utilized both suspensions [7] and isolated cadaveric fetal human retinal sheets [8].

Here we provide the required surgical essentials stratified by species and animal model to bring retinal derivatives of PSCs into the subretinal space in preclinical studies.

## 6.2 General and Anesthetic Considerations

### *6.2.1 Logistic and Management Advice*

The animals should be held indoors in a specialized facility in an air-conditioned room with temperatures between 18 °C and 20 °C, exposure to regular daylight and in standardized individual cages with free access to food and water [9].

To ensure the animals' operative affinity, an animal health score sheet is followed. This includes the following definitive animal exclusion criteria: 20% weight loss compared to weight on admission; inability to eat or drink; behavioral abnormalities such as CNS signs, vocalization, hunched posture, shivering, decreased activity, immobility; apparent cyanosis of the animal; has cramps or cannot move in

coordination; ataxia/paresthesia (e.g., paralyses); apathy; extreme automutilation (skin wounds, severed limbs).

All perioperative parameters should be carefully recorded and in case of abnormalities discussed with the surgeon and/or principal investigator in due time. Electronic record keeping and database filing is strongly encouraged. Animal IDs, such as tattoos, plastic tags, or even subdermal chips, should be considered, as they facilitate their identification during busy surgical days and follow-up examinations.

## *6.2.2 Animal Choices*

During the last 2 decades, there has been extensive research with animal models of retinal diseases. To date, several species—including mice, rats, cats, dogs, rabbits, pigs, and nonhuman primates—have been used as models to provide valuable information on the cellular and molecular aspects of pathogenesis of retinal diseases.

### 6.2.2.1 Small Rodents

The Royal College of Surgeons (RCS) rat is widely used for research as a hereditary retinal dystrophies model. It was identified to be a mutation in Mertk gene and results in defective retinal pigment epithelium phagocytosis of photoreceptor outer segments [10]. The RCS rat remains an obligatory disease model to evaluate the efficiency of RPE cell therapy in preclinical regulatory studies accepted by the US food and drug administration (FDA) to approve clinical trials for RPE cell therapeutics. However, several studies provided evidence that diverse cell populations other than RPE transplanted into RCS rats have also beneficial effects on ONL preservation [11–14]. Therefore, detailed analysis has to be performed to identify the RPE-specific effects on photoreceptor rescue in this model. Additional small rodent models used for long term teratogenicity assays were also the athymic nude rats [15] and humanized mice [16].

There is a naturally occurring mouse model of (retinitis pigmentosa-like) retinal degeneration, called the rd mouse, which exists in several variants [17]. Due to the often rapid retinal degeneration, the transplantation may need to be done at a very early stage [18], which makes it more challenging to operate on half smaller eyes compared to RCS rats [19].

### 6.2.2.2 Rabbits

We recommend Dutch Belted rabbits weighing a minimum of 1.5 kg due to less fibrin reaction than Chinchilla Bastard Hybrid rabbits [9]. Another alternative are New Zealand Reds, which are a pigmented crossbreed with New Zealand Whites

(albinos). The former have a very robust eye wall (rigid sclera) for retinal surgery and lesser bleeding tendency in the authors' experience. Albino rabbits (New Zealand White) are of limited value in our opinion, given aberrant RPE physiology and challenging contrasts of the retinal surface (due to the total lack of pigmentation), thus making subretinal manipulation difficult. One option to circumvent this challenge may be the use of chromovitrectomy dyes to stain the ILM such as triamcinolone or a commercially available mixture of trypan blue, brilliant blue G and PEG (Membrane Blue Dual® or Brilliant Peel Dual Dye ®).

#### 6.2.2.3 Pigs

The structure of the retinal vascular system in pigs is very similar to that of humans, which makes them very useful for research on eye diseases especially for retinal diseases.

Domestic Pigs

It is recommended to utilize smaller size domestic pigs (body weight 8–12 kg) to avoid management issues. Domestic pig growth rate is very fast as compared to mini and miniature pigs. The disadvantages of domestic pig models include lack of availability of molecular reagents and antibodies, difficulty in maintenance, and less precise genetic characterization and manipulations.

Mini-Pig

To develop effective cell therapeutics for RPE/Retina, it is imperative to establish an animal model that is reproducible, closest in anatomy to the human eye and most representative of the human disease. There is an increased interest to use mini-pigs in ocular experimental studies due to their anatomical similarities with human eyes and as a substitute for nonhuman primates. Pharmacologically, the mini-pig eye behaves similarly to the human eye, making it ideal for testing new therapeutics and surgical approach.

Yucatan Pig

Recently, there is an increased interest to use Yucatan pigs in ocular experimental studies due to their anatomical and physiological similarities with human eyes and as a substitute for nonhuman primates. Pharmacologically and physiologically, the Yucatan pig eye behaves similarly to the human eye, making it ideal for testing new therapeutics.

At the NEI, we use Yucatan minipigs weighing 30–35 Kg (around 6–8 months old). Yucatan's are preferred because of their fundus pigmentation; allowing us to use a micropulse 532 nm laser 48 h before surgery to damage the RPE in the visual streak (cone rich area) inducing a retinal degeneration. The laser induced retinal degeneration created allowed us to evaluate post-surgical retinal recovery (OCT, mfERG) in transplanted areas.

Recently, Ross et al. described a pig model of Retinitis pigmentosa with a Rhodopsin mutation, [20], thus offering a potent large-eye animal model for photoreplacement studies.

#### 6.2.2.4 Nonhuman Primates

Cynomolgus (*Macaca fascicularis*) and Rhesus monkeys (*Macaca mulatta*) weighing at least 3 kg and aged at least 2 years have been used by most investigators [21–25]. Some investigators used *Saimiri sciureus, a New World monkey* [26].

It appears as if rhesus macaques have a somewhat more robust foveal architecture for submacular surgery. Whenever feasible, the use of specific pathogen free (SPF) animals is recommended.

### *6.2.3 Immunosuppression*

#### 6.2.3.1 Mouse and Rat

Rodents were immunosuppressed with cyclosporine either by intramuscular injection (10 mg/kg) with cyclosporine blood level above 1500 μg/l [27] or when added (210 mg/l) to drinking water [28, 29], resulting in a mean cyclosporine blood level of 321 ± 21.9 μg/l [30]. Cyclosporine was given 1 day before transplantation until the end of the study. Lu et al. also included dexamethasone as an additional immunosuppressive drug by intraperitoneal injection (1.6 mg/kg/day) for 2 weeks after surgery [31]. Body weight and general physical condition of each animal were closely monitored every other day.

An alternative way is to use immunodeficient mouse disease model. Iraha et al. described two mouse models (NOG-rd1-2J and NOG-rd10) of end-stage retinal degeneration with immunodeficiency [32].

#### 6.2.3.2 Rabbit

Immunosuppression regimens in rabbits have been utilized extensively for RPE replacement. It is recommended to induce the animals at least 3 days before starting the surgery to avoid immune sensation already at surgery.

The most simple is perhaps the use of 1–2 mg intravitreal preservative-free triamcinolone under short-term intramuscular general anesthesia and local numbing drops (e.g., Oxybuprocaine), if few weeks of experimental follow up are planned [33, 34]. We discourage the use of systemic dexamethasone for subretinal RPE xenograft protection, as this resulted in disruption and/or cell loss within scaffold-supported RPE monolayer grafts [33]. To avoid steroids, both local [35] and systemic cyclosporine [7] protocols are available.

A triple immunosuppression regimen (prednisone, cyclosporine, and azathioprine) from the day of surgery throughout the experiment utilized by Del Priore [36] for aggregates of uncultured pig RPE sheets encapsulated in gelatin xenografted into the subretinal space of rabbits failed to provide protection at 4 and 12 week time points to both the graft and the host outer retina. This may be related to gelatin encapsulation being pro-inflammatory and was seen also by Stanzel et al. in human to rabbit RPE xenotransplantation experiments [33].

Our current recommendation is per oral Sirolimus (1 mg/day), Doxycycline (15 mg/kg/day) and Minocycline (15 mg/kg/day) started 3 days prior to surgery and continued throughout the experiment combined intravitreal triamcinolone at the time of surgery. The protocol is effective in protecting subretinal RPE xenografts in animals with a sodium iodate compromised outer blood–retinal barrier, see Fig. 6.1.

An intravitreal injection of Sirolimus (220 μg, DE-109, Santen Incorporated) [37] with a documented efficacy of up to 2 months in nonvitrectomized animals may obviate the need for repeated intravitreal injections or laborious systemic

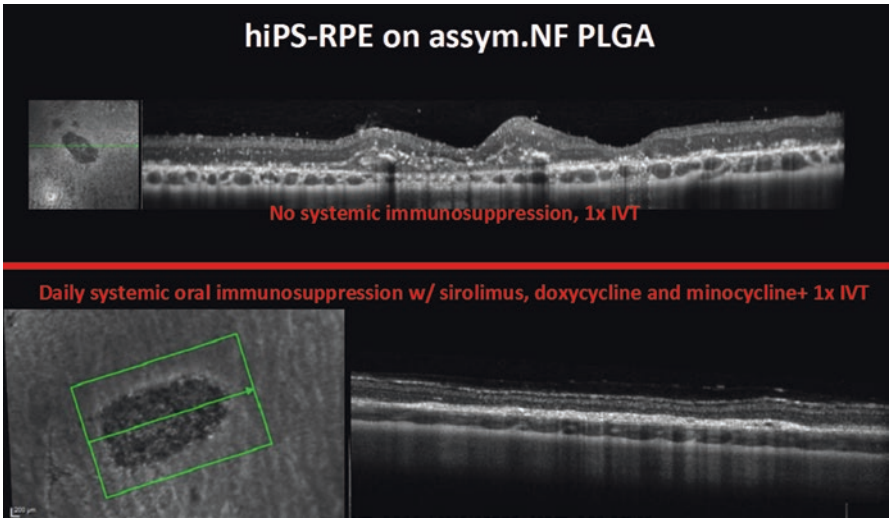


**Fig. 6.1** Effect of quadruple immunosuppression on subretinal human iPS-RPE xenograft integration in rabbits with damaged blood–retinal barrier

application. Here the drug typically deposits to the anterior vitreous and can be readily recognized as a whitish precipitate. Thus if during vitrectomy extensive vitreous removal is avoided, which is also safer to avoid lens touches, then the slow release formulation can be preserved in place, and may not necessitate repeated injections.

#### 6.2.3.3 Pig

Since scaffolds with human derived iPSC-RPE cells are introduced in the subretinal space, to minimize xenograft immune reaction, pigs are immunosuppressed starting 9 days before surgery and continued throughout until euthanasia. Tetracycline antibiotics doxycycline and minocycline are used orally with doses of 5 mg/Kg twice a day, mainly because of its suppressing effect on microglia activation. Steroids are used because of its broad-spectrum immunosuppressive effects. A loading dose of intramuscular methylprednisolone is used at doses of 5 mg/Kg, followed by similar daily oral single dose of prednisone. Both rapamycin (sirolimus) and tacrolimus are used to suppress adaptive immune response. Rapamycin is used orally with a loading dose of 2 mg, followed by a 1 mg daily dose. Tacrolimus is used in oral doses of 0.5 mg/day.

Koss et al. described pig model with perioperative sustained dexamethasone release implant (Ozurdex®, Allergan Inc.), along with a sophisticated continuous intravenous Tacrolimus application to protect monolayers of human embryonic derived RPE on porous parylene scaffolds, for further details, please refer to [38].

#### 6.2.3.4 Monkey

Systemic immunosuppression drugs in monkeys include sirolimus, doxycycline and minocycline. The animal should be immunosuppressed 7–10 days prior and throughout the experiment to avoid immune sensation at the time of surgery and immune rejection after surgery.

Systemic sirolimus is given per oral as follows: On first day 2 tablets of 1 mg/tab sirolimus once a day (2 mg/day), thereafter 1 tablets of 1 mg/tab once a day (1 mg/day) until enucleation. Difficulties in administering sirolimus orally (monkeys are picking and throwing the drugs even when disguised with gel, pudding or marshmallow) can be overcome by crushing the tablets and mixing into chocolate milk or if unavoidable, animals under light sedation (ketamine 5 mg/kg BW, IM). Doxycycline and minocycline dosage is 15 mg/kg and divided into twice daily per oral application in all animals of both cohorts. As an alternative fall back strategy systemic prednisone 5 mg/kg and tacrolimus extended release (Astagraf) 0.5 mg/day can be added to the above regimen in case control of over xenograft rejection reactions is not adequate.

## *6.2.4 Anesthesia*

### 6.2.4.1 Small Rodents (Mice and Rats)

Prior to starting anesthesia the animals are given food and water ad libitum and left in their own respective cage until needed. To ensure proper dosage of analgesics and anesthetics, the animal's weight is recorded. About 1–2 days prior to surgery pain management is started with Carprofen 5 mg/kg SC every 12–24 h or Metamizol 25 mg/drop in a solution 1:4, 0.2 to 2 drops every 6 h perorally.

Premedication is best achieved with a chamber for inhalation with isoflurane, alternatively an injection of Xylazine 5–10 mg/kg intraperitoneally can also be used. To avoid hypothermia you need a warm and soft pad throughout the procedure. Pupils are dilated by topical application of 1% tropicamide (1 drop) and a local anesthetic (to reduce dependence on general anesthesia), such as paraprocaine instilled on the ocular surface.

General anesthesia is then induced by intraperitoneal injection of a solution of ketamine 65 mg/kg (range 40–80 mg/kg), xylazine 13 mg/kg (range 10–15 mg/kg), and acepromazine 1.5–2 mg/kg; this will suffice for about 50 min. Observation of vital parameters, particularly hypothermia, is essential.

After the procedure the mouse/rat is taken to its own cage (isolation). The litter is covered with a tissue. Some food and possibly some gel cushions are placed on the bottom of the cage. The animal is closely observed until awake and has resumed eating and drinking. Thereafter regular follow up examinations of the operated eye, along with application of anti-inflammatory/antibiotic eye drops as per protocol are warranted.

### 6.2.4.2 Rabbit

General

Prior to anesthesia, the animal is allowed free access to food and water. Each rabbit is to be handled with care with good fixation of the rabbit, especially at the hind limbs since there is a high risk that they injure themselves. If the rabbit is agitated, it helps to place the palm of the hand over the ears (to pull them down to the side) and the extended index finger (of the same hand) onto the forehead, whilst gently pressing the head down. Once the rabbit has calmed down, it can be gently picked up by one hand sliding down to the scruff, whilst the other supports the bum (so the hind limbs do not hang free and start swinging/charging).

The animal's weight is recorded for anesthesia and analgesia dosage calculation. Pain management should be considered before, during, or directly after the surgery with, for example, meloxicam (2 mg/ml) 0.2 mg/kg SC every 24 h for 3–5 days.

The rabbit should be covered with a blanket to calm before the anesthesia injection. Premedication is achieved using intramuscular xylazine 0.2 mg/kg (IM)

injection into the hind limb (gluteal muscle) and massage around injection site for 30 s. A subcutaneous infusion (at the scruff) of 10 ml/kg 0.9% saline is recommended to stabilize hydration before and after surgery. For induction of anesthesia use ketamine 35 mg/kg IM and xylazine 3 mg/kg, and butorphanol 0.1 mg/kg. The maintenance solution containing ketamine 35 mg/kg IM, xylazine 3 mg/kg, and butorphanol 0.1 mg/kg should be injected 0.2 ml/kg every 20 min or when the rabbit shows wake-up reflexes. Alternatively, consider placing an intravenous line into the (well heated) auricular vein, if IV anesthesia is considered.

The first sign of the anesthesia fading away is nystagmus and must be monitored by the surgeon. The first shot of IM anesthesia lasts about 30–60 min, depending on the rabbit's size, drug tolerance, fat layer, stress, and body temperature. Always confirm proper anesthesia, by verifying hypnosis, hyporeflexia, analgesia, and muscle relaxation of the animal. Ensure the proper body core temperature using rectal thermometer (normothermia 39 ± 1 °C). A heating pillow underneath the rabbit may often not be necessary or even dangerous, if the animal is properly wrapped in blanket. An oxygen line placed under the blanket cover supplied with a funnel wrapping around the snout significantly helps to avoid accidental hypoxic brain damage during anesthesia. If feasible intraoperatively, observation of heartbeat and breath frequency can further improve anesthetic success.

After the procedure, the rabbit should be carefully transferred into warm cage with fresh hay, consider either a blanket or heating lamp, and positioned as specified by the surgeon (operated eye facing up or down). Do not leave the animal unattended until it regains sufficient consciousness to maintain sternal recumbence. Regular observation is warranted until the rabbit resumes fluid and food intake.

## Head Positioning

The optimal position of the rabbit under surgical microscope is with the nose slightly elevated through a mold of the blanket, so that it is level with ocular surface. Align the left eye perpendicular to microscope objective. We recommend using the left eye due to the positioning of the eye muscles. The eyelashes must be cut using scissors (some ointment on blade) to reduce postoperative infections.

## Eye-Specific Preparations

It is recommended to dilate the pupil prior to anesthesia with 2.5% phenylephrine and 1% tropicamide 1 h before surgery every 10 min, repeated three times; this will yield a faster and more lasting mydriasis. To disinfect the eye use 2–3 drops of 0.1 g/ml povidone–iodine topically for 1 min and rinse with sterile BSS. Then, the eye should be covered with sterile drape with a precut opening in the middle for the

eye and then covered with (sticky) surgical incision drape 12 × 17 cm. Methylcellulose or preferably a clinical grade dispersive viscoelastic lubricant should be added every 5–10 min to the operated eye, and lids in the nonoperated eye should be given lubricating ointment (e.g., Bepanthen®) and taped.

Postoperative eye care is recommended for 1 week twice a day, preferably with ointments containing a steroid and antibiotics (e.g., Isopto-Max®).

#### 6.2.4.3 Pig

General

The last feeding should be about 12 h before the start of the surgery, while access to water is not restricted. The weight of the animal is recorded for the dosage of anesthesia and analgesia. For sedation and to enable transport the pig to surgical suite for intubation inject intramuscular 5.0 mg/kg telazol, 0.2 mg/kg butorphanol, and 0.035 mg/kg dexmedetomidine. For premedication, 0.02 mg atropine/kg deep IM is given. Then the monitoring probes are placed on the animal.

General anesthesia is induced by inhalation of 2–5% isofluorane. To prevent eye drift and possibly fatal oculocardiac reflexes during surgery application of neuromuscular block Rocuronium at 2 mg/kg IV; injection is repeated as necessary.

Analgesia is administered while pig is still anesthetized, 0.005–0.01 mg/kg Buprenorphine IM. Pigs are sedated during recovery phase with 1.0 mg/kg Diazepam IV and 1.1 mg/kg Acepromazine IM, to prevent eye trauma.

Head Positioning and Eye Specific Preparations

Pigs are position in the surgical table in lateral recumbency and the head position so the cornea is perpendicular to the microscope objective. Eye lashes are trim and povidone-iodine 10% is used to clean the skin around the eye. Povidone–iodine 5% should be used for the cul-de-sac.

A single-piece drape with an integral fluid collection is the most efficient method of draping. Drapes without an opening for the eye should be used and a cut is made in the drape after it is adhesively applied with the lids open. The flaps created are then folded over the lid margins and kept in place by the lid speculum. To better expose the surgical area, a temporal canthotomy is made and a traction suture to proptose the eye is applied to the inferior rectus; if needed the nictitans membrane can be retracted with sutures to better expose the nasal sclera. The corneal epithelium should be constantly irrigated with BSS or (a dispersive) viscoelastic.

#### 6.2.4.4 Macaques/Nonhuman Primates

General

Presurgical preparation includes overnight fasting with no access to food from 6 pm onward to prevent regurgitation and vomiting during anesthesia and surgery.

In immediate preparation for surgery the monkey is sedated with intramuscular injection of Ketamine (10–20 mg/kg) and atropine 0.05 mg/kg (SC). An IM administration of Buprenorphine at 0.005–0.03 mg/kg BW (as pain reliever) is given 30 min to 1 h before surgery. The dose can be repeated 6 h after surgery, if necessary.

The animal should be weighed to ensure the accurate medication dosage. The 60–120 min procedure has to be performed under general anesthesia. A qualified veterinarian should perform intubation and general anesthetic procedure using 2% Isoflurane and an appropriately sized endotracheal tube according to the weight of the monkey. Vital signs such as electrical activity of the heart, respiratory rate, blood pressure, and oxygen saturation are tracked using monitoring equipment. The operation site is disinfected with alcohol, and finally swabbed with povidone iodine. Finally, the monkey is draped and ready for surgery.

During surgery, animals should receive vital signs monitoring including heart rate, respiration rate and/or end-tidal $CO_2$, $SpO_2$, temperature, and blood pressure.

Postprocedure analgesia is achieved with buprenorphine (0.005–0.1 mg/kg IM at 6- to 12-h intervals) or carprofen (2–4 mg/kg SC) for up to 3 days following all surgical procedures, and/or at any other time deemed necessary by the principal investigator or attending veterinary staff. The analgesia dosing depends on the animal's signs of pain (e.g., squinting, swollen eyelids, rubbing of the eyes, or tearing).

Animal Positioning

It is recommended to shave the periocular region to allow better access to the orbit and to improve adhesion of surgical draping. The optimal position of the monkey under surgical microscope is in supine position. To stabilize head position the use of a (custom-made) head mold is highly encouraged. Given occasional uncontrolled movements intraoperatively, we also suggest to secure the forehead with an adhesive tape which reaches to the surgical table. Ensure perfectly horizontal alignment of the limbal plane to ensure good surgical visualization and access to the macula. Retrobulbar injections to achieve a more proptosed position of the globe are very challenging.

Postoperatively, animals are then recovered in the recovery suite with the surgical eye stably positioned looking down for 1 h or longer to allow further reattachment of the macula.

Eye Specific Preparations

For pupil dilatation, the monkey should be given a mixture of 2.5% phenylephrine and 1% tropicamide eye drops prior to vitrectomy. To commence the procedure, tetracaine eye drops should be applied. Methylcellulose lubricant should be added every 5–10 min in the operated eye, and lids in the nonoperated eye should be taped. A sterile field is established by disinfection of periocular structures by exposure to 10% Povidone-Iodine and the ocular surface with 5% povidone–iodine for at least 1 min and rinse with sterile BSS. Thereafter, the animal has to be covered with a sterile drape and following placement of a lid speculum.

Postoperative application of Tobradex eye drops 5×/day and homatropine eye drops 3 times/day is followed for 1 week.

## 6.3 Subretinal RPE Graft Delivery

### *6.3.1 Mouse and Rat*

#### 6.3.1.1 Cell Suspension Preparation

Cultured RPE cells were trypsinized and resuspended (50,000 cells/μl) in BSS and loaded in a NanoFil syringe (RPE-kit, World Precision Instruments, USA) with 33-gauge bevelled needle. The needle was preflushed to avoid bubbles before injection.

#### 6.3.1.2 Cell Sheet Preparation

An RPE monolayer was cultured on scaffolds (e.g., porous polyester terephthalate/PET) for 4–8 weeks. Before the transplantation, implants (monolayer on top of scaffolds) were cut off with a fine blade or puncher with the size approximate 0.7 mm × 1.2 mm for RCS rats [39, 40] or 0.5 mm wide section for mouse [32]. The implants were loaded in a custom-made implant tool [28, 40, 41] and/or kept in BSS before implantation. We recommend these steps to be carried out under a microscope station with a sterile surface in a clean room or surgical suite.

#### 6.3.1.3 Animal Preparation Before Surgery

At the beginning of the surgery, the rats were placed on a sterile gauze under a surgical microscope. Eyes were dilated as described above. The skin around the eye was sterilized with 10% povidone–iodine and 1 drop of 5% povidone–iodine is applied to the eye surface followed 1 min later by a generous washout with BSS.

#### 6.3.1.4 Cell Suspension Injection

After the conjunctiva was cut open by a 33-gauge needle, a full-thickness cut through sclera was made 1.0–1.5 mm posterior to the limbus at the temporal equator of the host eye [30, 42], 0.5–2 μl BSS was injected at same side of the sclera-choroid opening into the subretinal space [29, 30, 42] or through the diametrically opposed retina with a blunt needle gently touching the retina [43]. The bleb was further verified by putting a glass coverslip on the cornea to visualize the fundus under surgical microscope.

#### 6.3.1.5 Cell Sheet Implantation

After the conjunctiva was opened, a small incision (approximately 0.8–1 mm for rats, 0.6–0.8 mm for mouse) was cut transsclerally approximately 1.5 mm posterior to the limbus at the temporal equator of the host eye by a 27 gauge needle until the choroid is exposed. A transcorneal anterior chamber paracentesis is performed by a 31-gauge needle to reduce IOP. A local retinal bleb is created by injecting approximately 5 μl BSS through the transscleral opening into the subretinal space by a 33-gauge or smaller blunt needle with a NanoFil syringe (RPE-kit, World Precision Instruments, USA). The bleb is further verified by putting a glass coverslip on the cornea to visualize the fundus under surgical microscope. The choroid was cut with a fine blade, while the retina remains intact. Occasional bleeding can be stopped with a cotton bud. The transplant is gently placed into the area of the subretinal bleb through the choroidal incision using a custom-made implantation tool [28, 40, 41] or 25 G intraocular forceps (with rigid scaffolds, such as PET). Optical coherence tomography (OCT) can be used to confirm the position of the implant immediately following the surgery. At the end of the surgery, the incision is closed with 10–0 Vicryl sutures.

### *6.3.2 Rabbit Surgery*

#### 6.3.2.1 Instrument Preparation

Establish and maintain a sterile field, by working in a closed room, wearing surgical scrubs, facial mask and hair cover. Disinfect hands prior wearing sterile surgical gloves. Place the sterilized instruments on a sterile drape. Place 1 ml syringe filled with 40 mg/ml 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. Also, 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 [9, 44]. The 27 G chandelier endoilluminator has to be connected to a light machine. Connect a vitrectomy set including

high speed vitrector and (Venturi) cassette to a vitrectomy machine. A 500 ml ophthalmic grade BSS bottle has to be connected to the cassette according manufacturer's instructions. Some vitrectomy machines will require priming of the system to be operational.

#### 6.3.2.2 Vitrectomy

First, make a lateral canthotomy and proptose and secure the eye with 3–0 silk using inverted caliper. Then, perform a conjunctival peritomy and incise the conjunctiva with Vannas' scissors close to the limbus, but far enough from the blood vessels (~1 mm distance). Dissect the conjunctiva by creating a "T-cut" by enlarging the peritomy with the scissor parallel to the limbus and then incise the conjunctiva vertically in form of a "T" for about 6–7 mm. Carefully separate conjunctiva/tenon by blunt dissection. A two-port, 25 G core vitrectomy can be performed through a non-contact, wide-angle system with a 27 G chandelier illumination system.

To create a sclerotomy, use a 25 G microvitreoretinal (MVR) blade or 25 G flat head trocar at 4 o'clock on left eye (OS) is carefully inserting the sharp tip of the blade in the direction toward the optic nerve. We recommend starting 3.5–4 mm behind the limbus. This will result in a transretinal rather than trans pars plana approach, but is thereby lens-sparing. Then, slowly retract the blade in the same direction and avoid enlarging the sclerotomy. Insert the custom side port-infusion cannula [45] and suture it using a 7-0 Vicryl suture and set the intraocular pressure (IOP) at 15–24 mmHg (at lower range if valved trocars with good seal are available). The second sclerotomy should be performed with a 27 G needle at 10 o'clock on OS. Then, insert a 27 G chandelier light into flat head trocar and fixate it with sticky tape and turn on the light source at ca. 20–30% (as per surgeon preference). To better visualize the ocular fundus, the corneal epithelium typically requires scraping with a surgical blade (judge by appearance of fine intraepithelial bullae).

Similar to the first sclerotomy, perform a sclerotomy at 2 o'clock on OS and (pre) place u-shaped 7-0 sutures around the sclerotomy without tying the knot if no trocars are used, otherwise secure the trocar with releasable sutures. Then insert the vitrectomy cutter tip by strictly pointing toward the posterior pole.

Start the vitrectomy around the entry port, to relieve vitreous traction at the transretinal entry site (see above) and ensure infusion flow. Meanwhile, 20 units/ml heparin and 0.001 mg/ml epinephrine should be added into the BSS infusion solution in parallel to reduce fibrin reaction. As heparin and epinephrine are not injected intraocularly, their effects are delayed depending on the infusion flow rate.

Then continue over the optic disc and the fibrae medullares using high speed vitrector by cutting the vitreous gel into small pieces at max 2000–8000 cuts/min and aspirating at max 100–200 mmHg [46]. A posterior vitreous detachment (PVD) has to be performed by separating the vitreous humor from the retina by holding the cutter over the posterior pole and (if feasible gently) superior of the optic disc, while aspirating only at max 50–200 mmHg without cutting (depending on how well sealed the system is, too much vacuum if eye wall collapse). The posterior hyaloid

face can be engaged in aspiration mode with the cutter, immediately inferior to the optic disc and then separated from the inner limiting membrane (ILM) using mechanical traction [9]. To visualize and facilitate (near total) removal of the floating vitreous over the posterior pole and midperiphery during vitrectomy inject ca. 50 µl (40 mg/ml) triamcinolone or diluted fluorescein (ca. 0.1 mg/ml) intravitreally and then thoroughly remove it with vitrectomy over the entire posterior pole. Avoid crossing over under the lens; if more peripheral removal is necessary we suggest using a third well sealing valved 25 G or 27 G trocar. Indentation to shave the peripheral vitreous (optionally by a skilled assistant) is recommended if gas or oil tamponade is desired; we recommend performing this step after the actual implantation procedure in an air-filled eye as the eye wall will become very unstable.

#### 6.3.2.3 Loading Implantation Instrument (Shooter)

Herein we describe the preparation of an instrument for subretinal implantation of RPE monolayer grafts on cell carriers; for subretinal injection of cell suspensions a commercially available 38 G Teflon tip cannula (MedOne) can be utilized. An air bubble to initiate the bleb retinal detachment and then, immediately following subretinal deposition of the RPE cell suspensions, another air bubble to seal the retinotomy, can facilitate the procedure [47]. For more details on loading the cannula with a suspension, the interested reader is referred to [8].

An RPE cell culture, regardless of source, should be rinsed prior to preparation of the implant three times with calcium- and magnesium-containing Hank's balanced salt solution (CM-HBSS).

A standard cell culture dish (100 × 20 mm) with 10 ml ophthalmic grade BSS should be centered under a light microscope. With a sharp, oval, custom-made hollow needle a 2.4 × 1.1 mm implant can be punched out to obtain a flat, bean-shaped substrate with two long edges and two round edges [9]. Then, gently flood the needle through the second port with BSS to flush out the implant. Optionally cut one round end of implant (<0.5 mm), just to obtain a third edge. Ensure that the implant is in the right orientation with the monolayer upside on the cell carrier. To change positioning carefully use two scalpels. Then, push the implant gently and completely into the shooter instrument using a needle holder until all of the implant is secured inside of the tip. The plunger should remain retracted and the "loaded" shooter tip should be kept under CM-BSS in dark until the moment of implantation.

#### 6.3.2.4 Implantation

Approach the neural retina with an extendible 38 G or 41 G subretinal injection needle connected to a gastight syringe, ensuring that all air bubbles have been evacuated from tubing. Then, create a bleb retinal detachment (bRD) (approximately 2–3 disc diameters) by slowly injecting 20–30 µl BSS subretinally by an assistant, with the IOP set to 25 mmHg or less. Two bRD per eye can be raised safely. The

same aforementioned procedure suffices to deliver a suspension of RPE, see above and [8].

A retinotomy enlarged to ca. 1.5 mm with a vertical 25 G VR-scissors is then created at the base of the 20–30 µl, so that the subretinal space is accessible for implantation or further maneuvering. The RPE underneath the bRD can be atraumatically scraped at high IOP (30–50 mm Hg), with a custom made 20 G extensible Prolene-loop instrument [44].

Before implantation, the sclerotomy at 4 o'clock (OS) must be enlarged (precisely) with a 1.4 mm incision knife to 20 G approach. Attempt passing through the sclerotomy with a 20 G shooter dummy and enlarge as needed to ensure smooth, yet snug transition of the loaded shooter. Then, pass with the loaded shooter through the sclerotomy at 15–25 mmHg [9]. Approach the retinotomy edge and eject the implant subretinally from an epiretinal position. The implant may be adjusted with half-closed 23 G scissors, forceps, or 38 G needle to make sure it is positioned well under the retina, away from the retinotomy. The RPE monolayer transplants should be placed cell-carrier-side down on bare Bruch's membrane or intact host RPE, ensuring that the xenografted RPE face photoreceptors. Drainage of subretinal fluid is optional, as the bRD will spontaneously resolve within few days or less [48]. If desired a slow-paced fluid-air exchange (FAX) with brush-tip silicone active extrusion cannula, rather than using perfluorocarbon liquids (PFC) based bRD flattening followed by FAX is preferable to avoid subretinal PFC entrapment. We discourage the use of expandable gases.

#### 6.3.2.5 Wound Closure

After removing 27 G chandelier and 25 G infusion cannula, suture all sclerotomies. Prior to suturing the last sclerotomy, inject 25–50 µl (40 mg/ml) triamcinolone intravitreally. The IOP should be checked by palpation and adjusted by injection of BSS via 30 G needle/syringe, if needed. Then, suture the conjunctiva with 7-0 vicryl and remove the proptosing 3-0 silk sling slowly. Avoid the deep orbital venous plexus as this may lead to uncontrollable hemorrhage. Concluding, suture the temporal canthotomy with 5-0 silk and add dexamethasone/antibiotic ointment under the lid.

### *6.3.3 Pig Surgery*

#### 6.3.3.1 Instrument and Equipment Preparation

Instrumentation and equipment depends on the surgeon's preferences. We use an Alcon Constellation® vitrectomy system and a Zeiss OPMI Lumera 700 microscope equipped with the Resight® noncontact fundus viewing system and Rescan 700 for intraoperative OCT (iOCT). The microscope is connected to the Alcon NGENUITY®

3D Visualization System for heads-up 3D visualization of surgical maneuvers. Hand instruments are best kept on a Mayo stand between the surgeon and the assistant with a back table placed behind for other surgical tools and disposables. A Mayo stand over the animal carries the vitrectomy probe, endolaser, bipolar handpiece, infusion and extrusion tubing. Powder free gloves should always be used.

#### 6.3.3.2 Vitrectomy and Induction of Retinal Detachment

A four-port pars plana vitrectomy is done 3.5 mm from the limbus. Only the nasal port conjunctiva is dissected to allow widening of the sclerotomy during implantation. Transconjunctival 25 G valved entry systems are used to ensure working in a closed system. To ensure sutureless ports, blades are introduced using a single plane 15° incision to create a scleral tunnel; only the nasal port blade is introduced perpendicular to the sclera with the flat edge in horizontal position to ensure a linear incision when widening the sclerotomy. The infusion port is located at the level of the external canthus and the infusion pressure set at 45 mmHg. The endoilluminator (chandelier) is located inferior nasally and 2 working ports (superior nasal and superior temporal) are made. A central and midperipheral vitrectomy is done using maximum cutting speed and aspiration (7500–10,000 cpm/650 mmHg). The lens should be spared so anterior vitreous cortex vitrectomy is avoided. A posterior vitreous detachment is induced using maximum aspiration beginning in the border of the optic nerve; if difficult, preservative-free triamcinolone acetonide is used to facilitate posterior vitreous identification. Triamcinolone is aspirated in the vitrectomy tip and released using the proportional reflux mode function in the foot pedal. A localized retinal detachment is produced in the laser-induced retinal degeneration area (visual streak) using an extendable PolyTip® 25/38 G cannula connected through a MicroDose™ injection kit (MedOne Surgical) to the viscous fluid injector system (VFI) set at 18 mmHg allowing precise food pedal control of the injected BSS. A 2.5 mm retinotomy is made with curved scissors. The temporal location of the infusion port prevents collapse of the retinal detachment due to turbulence when the nasal port is widened using a 2.4 mm slit knife (Mani®). We use a custom-made tissue clamp to close the enlarged sclerotomy while preparing for implantation.

#### 6.3.3.3 Implantation

Custom made injector design consists of hydraulically operated plastic ergonomic handle with an S-shape metal cannula and flattened plastic tip attached through the MicroDose™ injection kit to the VFI system where viscous fluid is replaced by a hyaluronic acid aqueous solution allowing for foot pedal control atraumatic delivery of implants into the subretinal space, see Fig. 6.2. This instrument accommodates a 2 × 4 mm oval shaped native tissue-like implant (iPSC-RPE monolayer on PLGA scaffold) that is cut from the transwell plate with a custom-made trephine. Transplanted cells are loaded in the injector manually using a syringe with hyaluronic

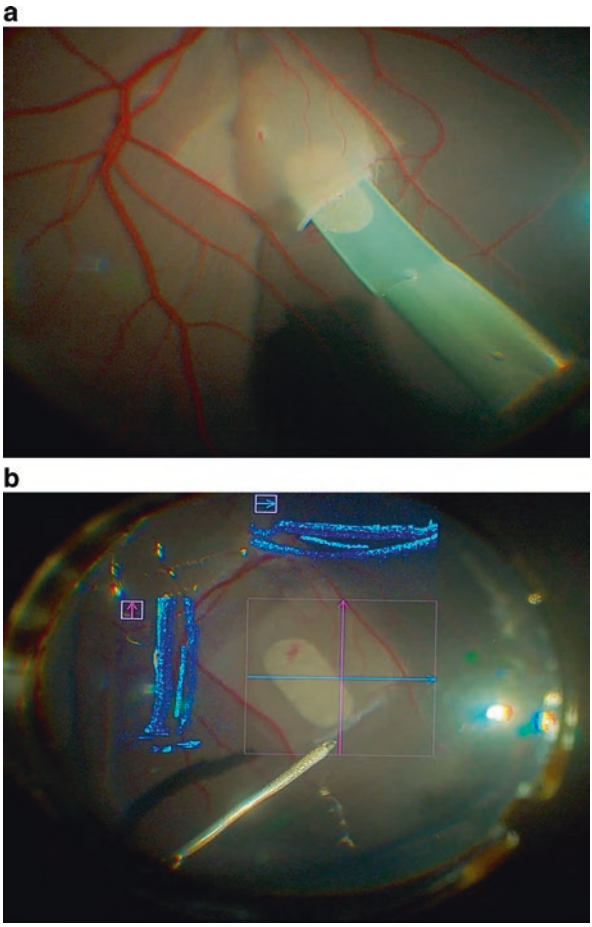


**Fig. 6.2** Subretinal implantation of a PLGA-scaffold supported iPS-RPE monolayer in pig. (**a**) Foot-pedal control subretinal release of iPSC-RPE PLGA patch from injector in micropulse induced retinal degeneration area (visual streak). (**b**) iOCT visualization of subretinal iPSC-RPE PLGA patch. Subretinal fluid is aspirated with extrusion cannula to flatten the retina while retinotomy is closed by apposition

acid aqueous solution connected to the MicroDose™ injection kit through a two-way valve [49].

#### 6.3.3.4 Ending Surgery

After implantation, the injector is released and the nasal sclerotomy temporally closed with the custom-made tissue clamp while fluid air exchange is used to reat-tached the bleb retinal detachment. The retinotomy is closed by apposition without retinopexy while iOCT confirms retinal flattening and implant location, see Fig. 6.2. The nasal sclerotomy is closed under air (nylon 8-0), air/gas exchange is made and ports withdraw. Always withdraw the infusion port at the end. Nasal conjunctiva is suture with Vicryl 7-0 and canthotomy with Vicryl 5-0. Administer subconjunctival 0.1 ml Depo-Medrol (20 mg/ml), 0.4 ml Gentamicin (100 mg/ml) followed with a

triple antibiotic ointment. Use an eye patch until the animal recovery. Ketoprofen 3 mg/Kg is used for 3 days and triple antibiotic ointment is applied BID for 5 days.

## *6.3.4 Monkey Surgery*

### 6.3.4.1 Instrument and Machine Preparations

Are identical to the general recommendations given for rabbits in Sect. 6.3.2.

### 6.3.4.2 Vitrectomy

First, perform a lateral canthotomy and insert a speculum. If globe exposure is insufficient, lids have to be retracted with traction sutures. Alternatively, a 360° conjunctival peritomy could be performed and a 3/0 silk sling suture is passed underneath rectus muscles to allow further exposure by suitable rotation of the globe. Preselecting (older) animals with good globe exposure can further facilitate surgical parameters.

Perform a conjunctival peritomy and incise the conjunctiva with a Vannas scissor close to the limbus, but far enough from the blood vessels. Dissect the conjunctiva by creating a "T-cut" by enlarging the peritomy with the scissor parallel to the limbus and then incise the conjunctiva vertically in form of a "T" for about 6–7 mm. The conjunctiva and Tenon are carefully separated from sclera by blunt dissection by the opening Vannas' scissor branches.

To perform a sclerotomy, use a 25 G microvitreoretinal (MVR) blade or 25 G trocar at 8 o'clock on right eye (OD) by carefully inserting the blade at 3 mm behind the limbus in direction toward the center of the globe. Then, slowly retract the blade in the same direction and avoid enlarging the sclerotomy. Insert a custom side port-infusion cannula and suture it using a 7-0 Vicryl suture and set the intraocular pressure (IOP) at 20 mmHg [45]. For a four-port vitrectomy, sclerotomies should be performed with a 25 G trocar at 2, 4, 8, and 10 o'clock on OD and preplace u-shaped 7-0 sutures around the sclerotomy with a releasable knot. Illumination is preferable with handheld endoillumination, rather than a trocar-based chandelier light source for improved maneuverability and light levels. The cornea should be lubricated and meticulously protected throughout the procedure with a mixture of ophthalmic grade viscoelastic (e.g., Viscoat®) and BSS; in case of corneal epithelial edema an abrasion with a surgical blade should not be deferred to improve fundus visualization (and surgical procedure time). Noncontact wide-angled fundus lenses attached to a surgical microscope (optionally equipped with an intraoperative OCT) are preferable for the rather small and often deep-set eyes.

Begin with core vitrectomy and vitreous removal around the main instrument ports. To visualize the vitreous fibers and facilitate surgical induction of posterior vitreous detachment inject ca. 20–50 µl (40 mg/ml) triamcinolone or diluted

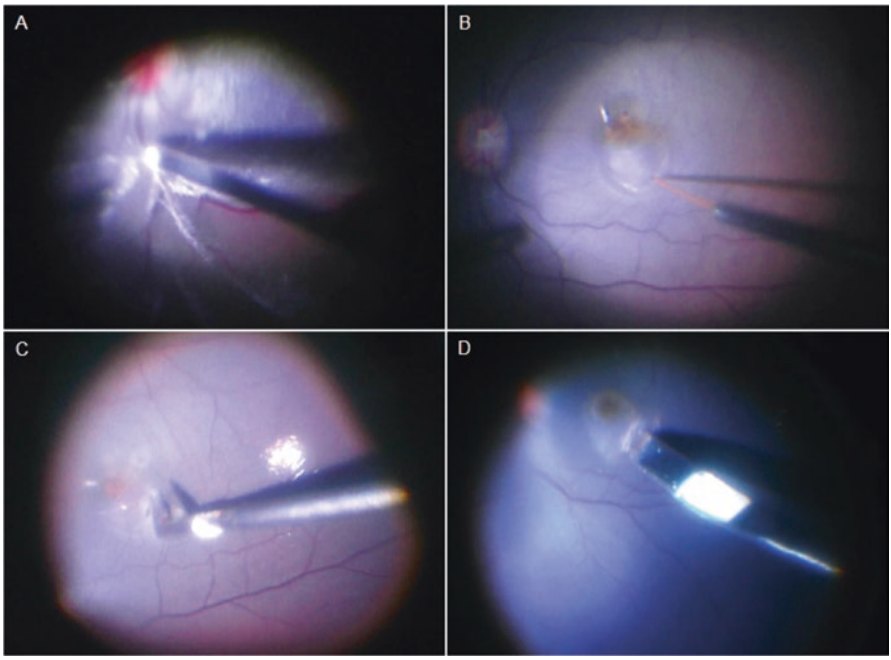


**Fig. 6.3** Submacular hES-RPE Implantation sequence in nonhuman primate. (**a**) Shows triamcinolone-aided detachment of posterior cortical vitreous from retinal surface, (**b**) shows subretinal BSS injection to induce detachment of the macula, (**c**) shows retinotomy temporal and distal to the immediate macular arteriole, (**d**) submacular implantation of hES-RPE monolayer on a porous polyester scaffold

fluorescein (ca. 0.1 mg/ml) intravitreally. The posterior vitreous detachment (PVD) is performed by separating the vitreous gel from the retina by holding the vitrector over the optic disc at maximum vacuum without cutting, see Fig. 6.3a. Avoid elevating IOP to values beyond 30 mmHg as this may result in inner retinal damage. If engagement of the posterior hyaloid face cannot be achieved with the vitrectomy cutter alone, then use an end-gripping forceps to create a tiny break in the cortical vitreous near the optic disc and repeat. Upon creating the PVD, remove the vitreous skirt up to the vitreous base.

#### 6.3.4.3 Preparing Implantation Instrumentation

Following same recommendation as in the rabbit section for scaffold supported RPE monolayer grafts. An alternative delivery technique for monolayer supported by its basement membrane was in part detailed (and tested in rabbits) by Kamao et al. [24, 50]. For RPE suspension grafts the recommendations are similar to what was given in the rabbit sect. 6.3.2.3, further details can be obtained by the interested reader in [22, 24, 51].

#### 6.3.4.4 Implantation

Approach and blanch the neural retina with an extendible 38 G subretinal injection needle connected to a gastight syringe (e.g., 100 µl Hamilton) with BSS, ensuring that all air bubbles have been evacuated from tubing. Then, create a gentle controlled bleb retinal detachment (bRD) by an assistant of about 2–3 disc diameter (DD) involving the macula with subretinal injection of BSS, see Fig. 6.3b.

Wilson et al. investigated the use instrumentation and route for subretinal injection of RPE cell suspensions in rhesus macaques. Following a trans-pars plana induction of a bRD, a 30 G cannula reproducibly delivered a suspension graft in the subretinal space via a trans-scleral approach [51].

For scaffold supported RPE monolayer grafts, a vertical 1.5 mm long temporal retinotomy is created distal to a temporal arteriole of the macula with a vertical 25 G VR-scissors at elevated IOP (ca. 30 mm Hg), so that the subretinal space is accessible for implantation or further maneuvering, see Fig. 6.3c. Diathermia should to be avoided if possible.

Before implantation, the trocar at the main right hand sclerotomy is removed, 7/0 vicryl sutures need to be preplaced, and the sclerotomy must be enlarged (precisely) with a 1.4 mm incision knife (MVR blade) to fit subsequent 20 G instrumentation.

The submacular RPE removal underneath the bRD can be achieved by scraping at high IOP with a custom made 20 G extendible Prolene-loop instrument [44]. Attempt passing through the sclerotomy with a 20 G shooter dummy and enlarge as needed to ensure smooth, yet snug transition of the loaded shooter. Meanwhile, the custom subretinal implant shooter need to be loaded by a sterile assistant as specified above. The graft loaded into the shooter instrument is then handed to the surgeon, who will pass it swiftly through the sclerotomy ideally at 20 mmHg to then eject the implant from epiretinal into the subretinal space. Upon removal of the shooter instrument, immediately close the main sclerotomy by preplaced 7-0 vicryl sutures to avoid ocular hypotension. Alternative approaches have been described for RPE monolayer grafts by Kamao et al. [24, 50].

The implant is preferably adjusted with the 38 G Teflon cannula (as it carries the least risk for Bruch's membrane injury) to make sure it is positioned well under the macula, away from the retinotomy. The RPE monolayer transplants should be placed cell-carrier-side down on bare Bruch's membrane with the xenografted RPE facing photoreceptors.

Fluid air exchange is performed via active extrusion through the left hand trocar (or newly inserted right hand trocar) using a brushed silicone soft tip cannula. Gentle subretinal fluid aspiration from the bleb retinal detachment and retinotomy edge apposition is attempted at very low vacuum settings. Laser retinopexy to stabilize the retinotomy edges should be avoided. All steps of the submacular implantation may be monitored or guided if possible with intraoperative optical coherence tomography (iOCT). The left hand trocar port can be then used to for 1–2 mg preservative free triamcinolone (e.g., Triesence®) instillation.

#### 6.3.4.5 Wound Closure

After removing 25 G chandelier and 25 G infusion cannula, remove all trocars and suture the sclerotomies. The IOP should be checked by palpation and adjusted if needed. Suture the conjunctiva with 7-0 vicryl and the temporal canthotomy with 5-0 silk. Polymyxin/neomycin/bacitracin antibiotic ointment is administered into the conjunctival sac.

## 6.4 Photoreceptor Transplantation

### *6.4.1 Considerations for Photoreceptor Graft Preparation*

Retinal sheet transplantation offers the possibility of targeting both photoreceptors and RPE degeneration, and it can also consistently achieve a large continuous layer of photoreceptors with outer segments in contact with host or donor RPE, in recipients with a severely degenerated photoreceptor layer [52]. Due to the availability of stratified retinal tissue generated from pluripotent stem cell-derived retinal organoids, first studies provide evidence for proper orientation and polarization of in vitro-generated donor transplants including RPE contacts and synapse formation to endogenous second order neurons resulting in some functional improvements in the blind rd1 mouse model [53]. However, sheet transplantation approaches also show several limitations, when compared with the injection of suspended cells. The procedure is difficult and requires extensive training, as well as the acquisition of custom devices to deliver the sheet. The isolation of pure photoreceptor sheets from fetal or retinal organoid sources remains challenging, as in most cases inner retinal cell populations, that is, bipolar, horizontal, or amacrine cells besides Müller glia, are still attached to the photoreceptors, thus interfering with proper graft–host connectivity. Furthermore, transplantation of sheets frequently leads to rosette formation within the photoreceptor layer thus disrupting photoreceptor—RPE interactions, resulting in limited outer segment phagocytosis and consequently reduced chromophore recycling [53].

In contrast, the transplantation of dissociated cells, despite also requiring extensive training to master the subretinal injection procedure, has the advantage that only a small, self-healing incision is necessary for cell delivery, thus reducing tissue trauma. A minimal invasive procedure will be of particular importance in patients with advanced retinal degeneration, as such disease stages are associated with significant tissue thinning, which increases potential complications, such as retinal ruptures or complete retinal detachment. While preclinical studies provided evidence for proper maturation of donor photoreceptors, including synapse and outer segment formation following suspension injections, donor suspension transplants mainly generate disorganized cell clusters in the subretinal space, lacking a strict apical-basal orientation [54, 55].

### 6.4.2 Photoreceptor Transplant Delivery Strategies

The delivering technique of donor photoreceptors is another factor that significantly impacts the efficiency of transplantation. Subretinal transplantation in rodents can be performed by two alternative routes: transscleral and transvitreal injection, see Figs. 6.4 and 6.5.

While the trans-scleral approach allows relatively easy accessibility to the subretinal space (Fig. 6.4), visibility during the injection is reduced. If the instrument nozzle is placed at the wrong angle, it can damage Bruch's membrane, disrupting the RPE and the blood–retinal barrier (BRB), which in turn may cause bleeding and/or infiltration of the transplant with lymphocytes and macrophages, thus leading to transplant loss or rejection.

Transvitreal injections offer a clear visualization of the injection site and of the blood vessel network, thus reducing the risk of hemorrhage (Fig. 6.5). However, it induces some local retinal gliosis when the needle pierces through the retina.

Two methods for cell delivery in the subretinal space have been proposed: the transplantation of whole retinal sheets and the injection of suspended disaggregated cells. Retinal sheet transplantation methods were first developed in the 1990s for the delivery of gelatin embedded donor tissue, which is rolled up to fit into a round nozzle, and then unfolded after insertion into the subretinal space [56]. This method requires the formation of a subretinal bleb, as well as significant amounts of fluid to deliver the sheet, which causes trauma to both host and donor tissue. Retinal reattachment after delivery is also necessary. An improvement over this method was proposed later, in which a custom implantation device allowed a gentle placement of the retinal sheet, in the correct orientation and with minimal amount of fluid [57]. This variation does not require retinal reattachment and has been shown to produce cell integration within a degenerating retina [58].

### 6.4.3 Future Outlook for Photoreceptor Replacement

While photoreceptor transplantation is currently still at the preclinical stage, it is expected that, with the acquired knowledge in subretinal delivery of stem cell-derived RPE in first patients [59–62], clinical transplantation of photoreceptors will be performed within the coming years. Improved phenotyping of the degenerating retina with defining specific disease stages will be of utmost importance to identify the proper surgical method for donor cell delivery. Besides the degree of photoreceptor loss, additional environmental factors within the retina, like glial reactivity and scar formation, RPE constitution, hemorrhage, and inflammation might have significant influence on transplantation success.

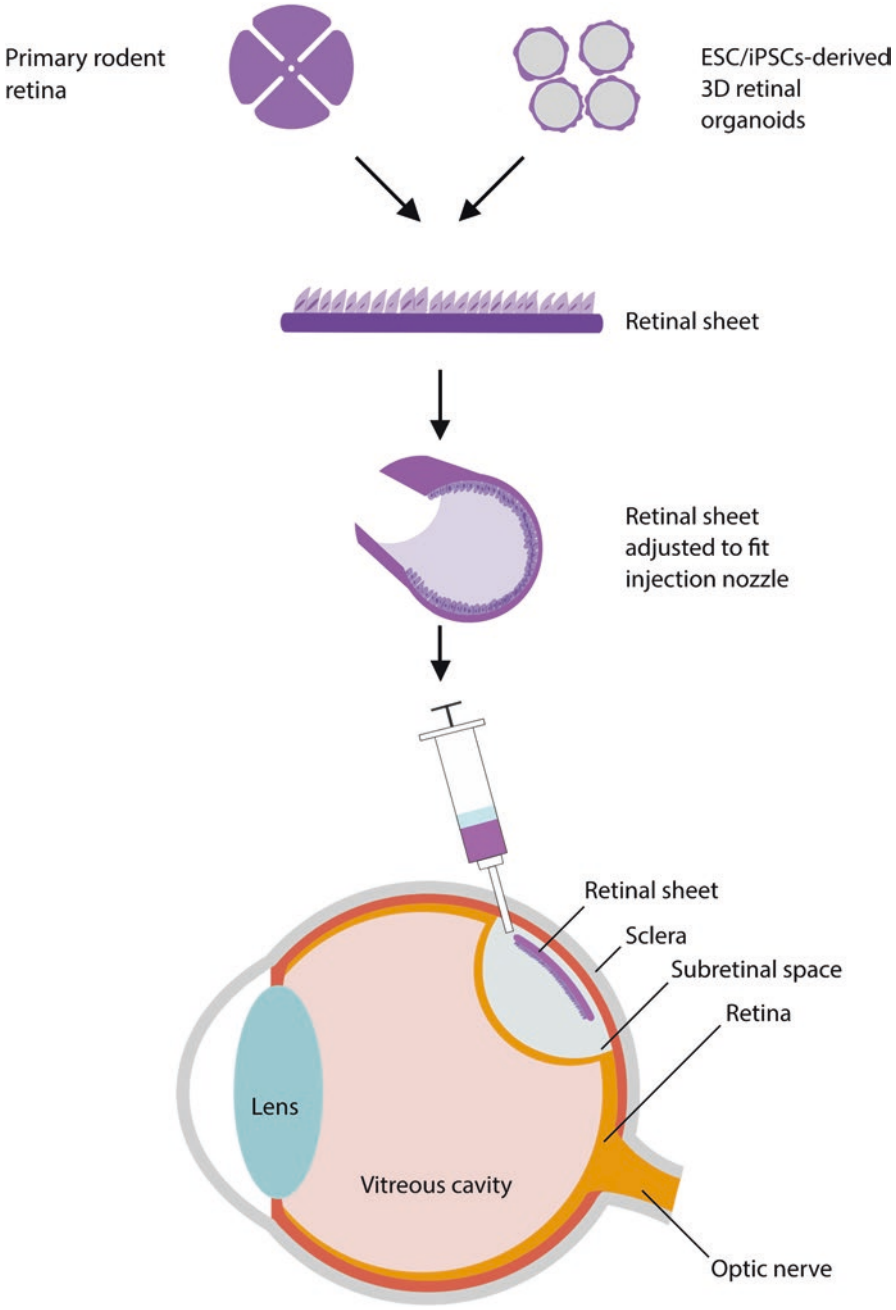


**Fig. 6.4** Transscleral delivery of retinal transplants

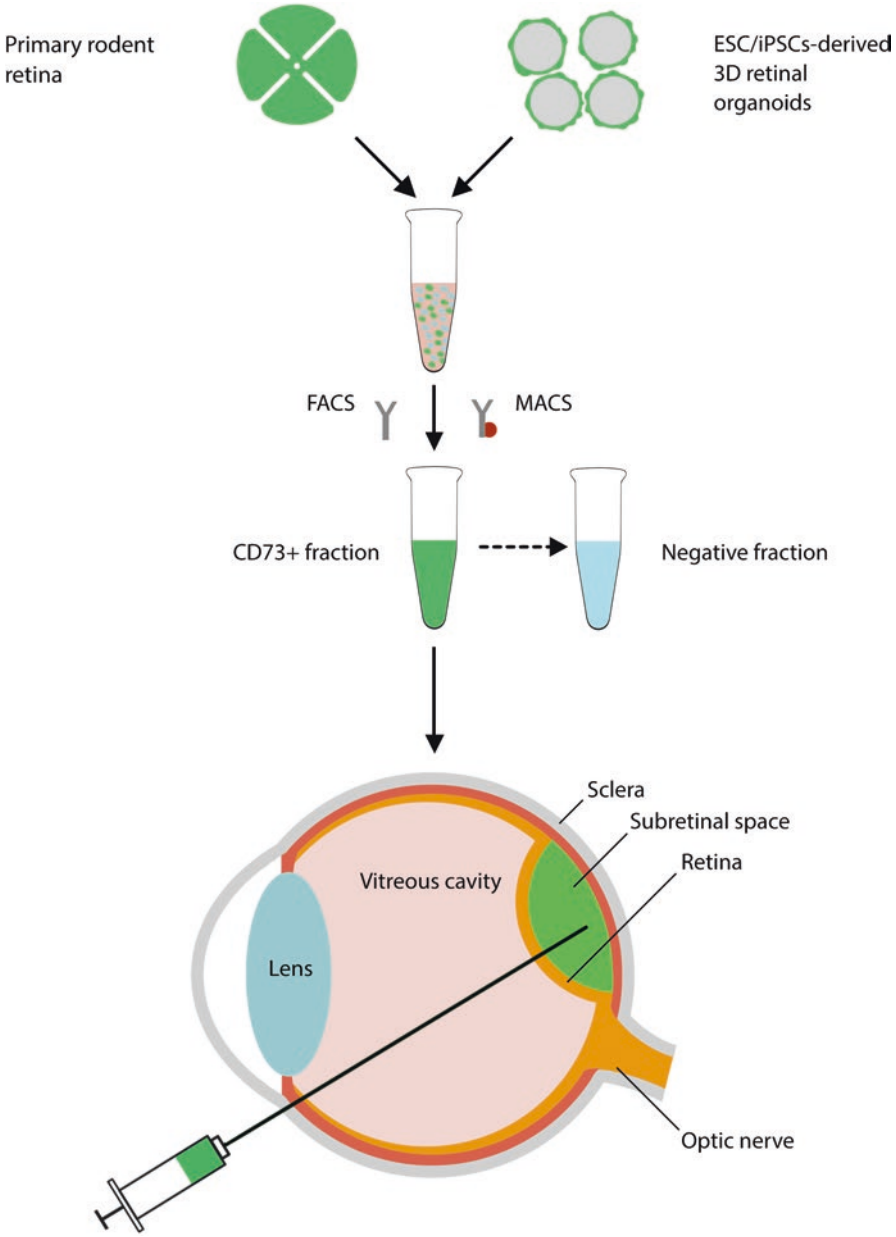


**Fig. 6.5** Transvitreal delivery of retinal transplants

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