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TITLE:
Subretinal Transplantation of Human Embryonic Stem Cell-Derived Retinal Tissue in a Feline Large Animal Model

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KEYWORDS:
retinal organoids, cat, large eye animal model, subretinal transplantation, retinal degeneration, surgical technique

SUMMARY:
Presented here is a surgical technique for transplanting human pluripotent stem cell (hPSC)-derived retinal tissue into the subretinal space of a large animal model.

ABSTRACT:
Retinal degenerative (RD) conditions associated with photoreceptor loss such as age-related macular degeneration (AMD), retinitis pigmentosa (RP) and Leber Congenital Amaurosis (LCA) cause progressive and debilitating vision loss. There is an unmet need for therapies that can restore vision once photoreceptor has been lost. Transplantation of human pluripotent stem cell (hPSC)-derived retinal tissue (organoids) into the subretinal space of an eye with advanced RD brings retinal tissue sheets with thousands of healthy mutation-free photoreceptors and has a potential to treat most/all blinding diseases associated with photoreceptor degeneration with one approved protocol. Transplantation of fetal retinal tissue into the subretinal space of animal models and people with advanced RD has been developed successfully but cannot be used as a routine therapy due to ethical concerns and limited tissue supply. Large eye inherited retinal

degeneration (IRD) animal models are valuable for developing vision restoration therapies utilizing advanced surgical approaches to transplant retinal cells/tissue into the subretinal space. The similarities in globe size, and photoreceptor distribution (e.g., presence of macula-like region *area centralis*) and availability of IRD models closely recapitulating human IRD would facilitate rapid translation of a promising therapy to the clinic. Presented here is a surgical technique of transplanting hPSC-derived retinal tissue into the subretinal space of a large animal model allowing assessment of this promising approach in animal models.

INTRODUCTION:

Millions of people around the world are impacted by retinal degeneration (RD) with resulting visual impairment or blindness associated with loss of the light-sensing photoreceptors (PRs). Age-related macular degeneration (AMD) is a major cause of blindness resulting from a combination of genetic risk factors and environmental/lifestyle factors. In addition, over 200 genes and loci have been found to cause inherited RD (IRD)[1]. Retinitis pigmentosa (RP), the commonest IRD, is genetically heterogenous with more than 3,000 genetic mutations in approximately 70 genes being reported[2,3,4]. Leber Congenital Amaurosis (LCA), which causes blindness in childhood is also genetically heterogenous[5,6]. Gene augmentation therapy has been developed and is in clinical trials for treating a small number of IRDs[3,7]. However, a separate therapy must be developed for the treatment of each distinct genetic form of IRD and thereby only treating a small subset of patients. Furthermore, gene augmentation relies on the presence of a population of rescuable photoreceptors and is, therefore, not applicable for advanced degeneration.

There is, therefore, an urgent and yet unmet clinical need for the development of therapies addressing and treating advanced RDs and profound to terminal blindness. Over the last 2 decades neuroprosthetic implants have been developed and tested in large animal models, such as the cat, prior to human use[8-14]. Likewise, in the past 20 years retinal replacement therapies utilizing sheets of embryonic or even mature mammalian retina grafted subretinally have been developed[15-22] and even tested successfully in RD patients[23-25]. Both approaches utilize the idea of introducing new sensors (photovoltaic silicon photodiodes in the case of neuroprosthetic devices[26,27], and healthy mutation-free photoreceptors organized in sheets, in the case of retinal sheet implantation) into retina with degenerated PRs. Recent studies have investigated the use of stem cells-based approaches such as transplantation of human pluripotent stem cell (hPSC)-derived retinal progenitors[28,29], hPSC-photoreceptors[30], and hPSC-retinal organoids[31-33]. Retinal organoids enable the formation of retinal tissue in a dish and derivation of photoreceptor sheets with thousands of mutation-free PRs, which resemble the photoreceptor layer in the developing human fetal retina[34-40]. Transplanting hPSC-derived retinal tissue (organoids) into the subretinal space of patients with RD conditions is one of the new and promising investigational cell therapy approaches, being pursued by a number of teams[31,32,41,42]. Compared to transplantation of the cell suspension (of young photoreceptors or retinal progenitors), transplanted sheets of fetal photoreceptors were demonstrated to result in vision improvements in clinical trials[23,24].

The protocol presented here describes, in detail, a transplantation procedure for subretinal delivery of the whole retinal organoids (rather than organoid rims[33,41]) as a potentially better way

to introduce intact retinal sheets with PRs, to increase graft survival and improve the sheet preservation. Though procedures for introducing a flat piece of human retina and also RPE patches have been developed[43-45], transplantation of larger 3D grafts has not been investigated. Stem cell-derived retinal organoids provide an inexhaustible source of photoreceptor sheets for developing vision restoration technologies, are free of ethical restriction, and are considered an excellent source of human retinal tissue for therapies focused on treating advanced RD and terminal blindness[46]. Development of surgical methods for precise subretinal implantation of retinal organoids with minimal injury to the host retinal niche (neural retina, retinal pigment epithelium and retinal and choroidal vasculature) is one of the critical steps for advancing such therapy toward clinical applications[31,32]. Large animal models such as cats, dogs, pigs, and monkeys have proven to be good models for investigating surgical delivery methods as well as to demonstrate the safety of implanted sheets of tissue (retinal pigment epithelium (RPE) cells) and investigate the use of organoids[41,44,45,47-50]. The large animal eye has a similar globe size to human as well as similar anatomy including the presence of a region of high photoreceptor density, including cones (the *area centralis*), resembling the human macula[6,51,52].

In this manuscript, a technique for the implantation of hPSC-derived retinal tissue (organoids) into the subretinal space of feline large animal models (both wild-type and *Crx*$^{Rdy/+}$ cats) is described, which, together with promising efficacy results[32,53] builds a foundation for further development of such investigational therapy toward clinical applications to treat RD conditions.

PROTOCOL:

Procedures were conducted in compliance with the Association for Research in Vision and Ophthalmology (ARVO) statement for Use of Animals in Ophthalmic and Vision Research. They were also approved by the Michigan State University Institutional Animal Care and Use Committee. Wild-type and *Crx*$^{Rdy/+}$ cats from a colony of cats maintained at Michigan State University were used in this study. Animals were housed under 12 h : 12 h light-dark cycles and fed a commercial complete cat diet.

1. Pre-implantation procedures and surgical set-up

1.1. Select wild-type or *Crx*$^{Rdy/+}$ cats depending on the study design. Perform a pre-surgical ophthalmic examination, including slit lamp biomicroscopy and indirect ophthalmoscopy. Exclude any animals with fundus abnormalities not related to their genotypes.

1.2. One week prior to implantation, start the animals on an immunosuppressive protocol of oral cyclosporine 2 mg/kg and prednisolone 1 mg/kg both twice daily to help prevent transplant rejection.

1.3. Fast the animals over 4 months of age overnight (at least 8 h). Provide a limited amount of wet food overnight to the animals less than 4 months of age, and then, fast them for 2 h prior to the surgery.

1.4. Perform a general physical examination, including chest auscultation (using a stethoscope). Record the heart and respiratory rate, temperature, mucous membrane color, and capillary refill time.

1.5. Pre-medicate the animals less than 4 months of age with buprenorphine (0.02 mg/kg), and those over 4 months of age with buprenorphine (0.02 mg/kg) combined with acepromazine (0.02 mg/kg) subcutaneously or intramuscularly 30–45 min prior to general anesthesia induction.

1.6. Apply topical 1% tropicamide ophthalmic solution and 10% phenylephrine ophthalmic solution to the ocular surface at least twice to dilate the pupils. Repeat if pupils are not well-dilated.

1.7. Place a 22 G intravenous catheter in the cephalic vein in all the animals: first clip hair from a 2 x 3 cm area over the cephalic vein; prepare the skin by first scrubbing it with 70% ethanol and then with a chlorhexidine scrub; place the catheter and secure with medical tape and flush with heparinized saline. In animals under 4 months, this can be done following induction of anesthesia.

1.8. Induce general anesthesia 30–45 min after premedication: animals under 4 months of age are induced with isoflurane delivered by mask, those over 4 months of age are induced using intravenous propofol (4–6 mg/kg).

1.9. Intubate with an appropriate size of endotracheal tube. Visualize the larynx with an examination light or laryngoscope. Spray 0.1 mL of lidocaine 2% on to the larynx, wait for a few seconds and then intubate.

1.10. Maintain anesthesia with isoflurane (between 2%–3.5%) in oxygen (300–600 mL/kg/min) via a Bain system.

1.11. Place the animal in dorsal recumbency on a positioning pillow on which a heated water blanket covered by towels is positioned. Attach a patient monitor and monitor the heart rate and electrocardiogram (ECG), respiratory rate, blood pressure, oxygen saturation, and end-tidal carbon dioxide. Regularly monitor the body temperature during the procedure. A suitably trained person is responsible for the maintenance and monitoring of anesthesia.

1.12. Position the animal with the help of the positioning pillow such that the corneal surface is horizontal when the eye is rotated into a primary gaze position. Secure the head in place using medical tape.

1.13. Cover the animal with blankets to help maintain the body temperature.

1.14. Flush the intravenous catheter with heparinized saline and start an intravenous infusion of Ringer lactate supplied at 2–5 mL/kg/h for the duration of the procedure.

1.15. Prepare the ocular surface, conjunctival sac, and eyelids for aseptic surgery using 0.2% povidone-iodine, sterile cotton swab applicators, and cotton balls.

1.16. Position the operating microscope with eyepieces also adjusted appropriately. Place the foot control for the microscope (focus, zoom, and XY-axis control) and vitrectomy machine such that the surgeon can operate them.

1.17. Prepare for aseptic surgery: all personnel must wear surgical scrubs, a surgical hat, and a mask. Ensure that the surgeon and the assistant(s) scrub, gown, and glove as for routine aseptic surgery. All personnel should be familiar with aseptic techniques.

1.18. Once the personnel are scrubbed, gowned, and gloved, open the surgical packs, and lay out the instruments. Place sterile manipulation knobs on the microscope adjustment knobs to allow the surgeon or the assistant to adjust without breaking asepsis. Drape the animal in a routine fashion for ocular surgery.

1.19. Prepare the vitrectomy machine following the manufacturer's instructions for a two-port vitrectomy.

1.19.1 Place a vitrectomy contact lens, a two port 23 G vitrectomy on the assistant's table. Attach the wet field cautery. Attach and prime the infusion tubing and the 23 G vitrectomy handpiece.

1.19.2. Place the vitrectomy instruments and fluid lines over the sterile drape and maintain them in position using towel clamps. Set the vitrectomy machine in Proportional Vacuum mode between 1,500 and 2,500 cuts per minute (cpm) and maximum vacuum of 500 mmHg. This is performed by pressing on the arrow buttons (upward or downward) present on the front panel of the vitrectomy machine.

2. Preparation of the organoids for subretinal implantation (Figure 1)

2.1. Derive retinal organoids as outlined earlier[31], and, if needed, ship overnight at 37 °C as previously reported[54].

2.2. Wipe down the surfaces in the tissue culture room in the receiving laboratory with a disinfectant and maintain the same high level of aseptic technique as in a surgical suite for ocular surgeries, to avoid bacterial or fungal contamination, and carry over into the surgical room to avoid surgical site infection.

2.3. Wear surgical shoe covers, disposable lab coats, bonnets, sleeves, and surgical scrubs inside the tissue culture room assigned for organoid preparation for ocular surgeries.

2.4. Pre-equilibrate neural media with 20 ng/mL of basic fibroblast growth factor (bFGF) and 20 ng/mL of brain-derived neurotrophic factor (BDNF) for 1 h in a tissue culture incubator (37 °C, 5% CO_2). Place organoids in media in ultra-low adhesion plates using approximately one-fourth

volume of conditioned medium (from the overnight shipment) and three-fourth volume of fresh medium[31,54]. Maintain for 24–48 h.

2.5. Prepare and equilibrate several fresh 60 mm plates with neural medium (as prepared in step 2.4) for at least 1 h in the tissue culture incubator (37 °C, 5% CO_2) before anesthetizing the first animal. Note, these plates are used for transferring the organoids for each individual surgery, with an assumption that pH conditions and CO_2 saturation will be maintained in the media for at least 20 min, sufficient for moving the plate to the surgical room and loading organoids into the cannula.

2.6. Use a fresh 60 mm plate with pre-saturated neural media for each transplantation case, while keeping the remaining plates in the tissue culture incubator at 37 °C.

2.7. Transfer 6–9 organoids into one 60 mm dish (with re-saturated media) by pipetting them out with a manual single-channel pipette (20–200 µL) with 200 µL sterile filter tip having a wide (~0.7 mm) opening. These tips can be prepared in advance or during the procedure by snipping ~3-4 mm of the tip with a pair of sterile scissors (done in the tissue culture hood to avoid contamination). Place the 60 mm dish inside a 100 mm tissue culture dish (to avoid contamination during room-to-room transport) and quickly transport to the surgical suite.

2.8. Place the plate with organoids on a 37 °C electric heating pad covered by a sterile drape.

2.9. Change into a fresh pair of sterile gloves before prepping the organoids.

2.10. Rinse the organoids in sterile balanced salt solution (BSS) (optional), and then load into the injector, which consists of a glass cannula (outer diameter, OD 1.52 mm; inner diameter, ID 1.12 mm) with a polished blunt end attached by sterile plastic tubing to a sterile 500 µL Hamilton syringe pre-filled with sterile BSS.

2.11. Pass the cannula to the surgical team in a sterile fashion when they are ready to inject the organoids.

2.12. Keep the length of the entire procedure (from removing the organoids from tissue culture media to loading the cannula) to 20 min or less.

2.13. If there is a delay in implanting the organoids, place them back into the tissue culture incubator, to avoid changes in pH/CO_2 saturation inside the dish.

2.14. Retain unused organoids for 1 week in the same tissue culture incubator and monitor for any evidence of contamination.

3. Subretinal organoids implantation

3.1. Perform a 0.5–1 cm lateral canthotomy with Stevens tenotomy scissors. Place an appropriately sized Barraquer eyelid speculum to keep the eyelids open. Ensure that the surgical assistant irrigates the cornea regularly with BSS for the duration of the procedure.

3.2. Place 2 stay sutures of 6–0 silk suture in the conjunctiva immediately adjacent to the limbus at the 4 and 8 o'clock positions to hold the eye in primary gaze and retract the third eyelid. Use 0.5 Castroviejo corneal tying forceps and a small mosquito hemostat to gently grasp the bulbar conjunctiva next to the limbus. Leave the sutures long and clamp the ends with small mosquito hemostats to help with their manipulation.

NOTE: Placing the suture under the third eyelid is more difficult; be sure to place it immediately adjacent to the limbus.

3.3. Place another suture at the 12 o'clock position at the limbus partially through the thickness of the limbus taking care not to penetrate the eye. Knot this suture loosely and cut the ends short. This provides a robust anchor suture, which is used during the surgery as a "handle" to rotate the eye to allow the surgeon access to different parts of the globe during different stages of the procedure.

NOTE: Steps 3.2 and 3.3 can be inverted. The sequence of stay sutures placement is the surgeon's preference.

3.4. Reflect the bulbar conjunctiva between 10–2 o'clock (a perimetry). Using tenotomy scissors, incise the conjunctiva 2–3 mm from the limbus. Undermine it and clear the tenon's capsule to expose the sclera at the sites for the 2 and 10 o'clock vitrectomy ports, which will be placed about 3–5 mm from the limbus depending of the age of the animal. Use surgical cellulose spears for hemostasis and to clear any blood.

3.5. Identify sites for sclerotomy following the reflection of the conjunctiva and tenon's capsule using calipers. Choose sclerotomy sites (at 2 and 10 o'clock aiming to pass through pars plana), to avoid the major scleral vessels that can be prominent in the cat. Use wet-field cautery on the sclera at the planned sclerotomy sites to reduce scleral bleeding, planning for an ~3 mm region at the instrument port (10 o'clock port for a right-handed surgeon) as this will be enlarged following the vitrectomy to allow the implantation cannula to be introduced.

3.6. Pre-place cruciate pattern sutures (6-0 or 7-0 polyglactin suture) without tying the knot at the site of the proposed sclerotomies before the vitrectomy ports are placed.

NOTE: This facilitates rapid closure of the sclerotomies at the end of the procedure. The suture for the planned instrument port needs to have a longer bite of sclera as this will be a long incision.

3.7. Once the sutures are placed, ask the assistant to help position the globe by holding the 12 o'clock stay sutures by tying or using Bishop-Harmon forceps. Let the surgeon stabilize the globe as well, using a 0.12 mm Castroviejo forceps to hold the tissue next to the sclerotomy site.

3.7.1. Introduce a 23 G vitrectomy port using a trocar through the sclera at both the 2 o'clock and 10 o'clock positions directed at an angle toward the optic nerve to avoid contacting the lens.

3.7.2. Check that the irrigation port is in the vitreous by gently pushing on the port using tying forceps so that the tip can be visualized in the vitreous. Once correct positioning is confirmed, attach the irrigation line to the port and position the line using thin adhesive bandages to tape it in place. Set the vitrectomy infusion of the BSS buffer initially at 30–35 mmHg by pressing on the arrow buttons (upward or downward) present on the front panel of the vitrectomy machine.

3.8. Let the assistant hold a Machemer magnifying irrigating vitrectomy lens onto the cornea to allow visualization of the posterior segment of the eye during the next stages. Attach the irrigating vitrectomy lens to a drip set providing constant fluid supply to couple the lens to the cornea.

NOTE: Other forms of contact vitrectomy lens could also be used. Dim or switch off the room lights to help visualization through the operating microscope.

3.9. Insert the 23 G vitrectomy probe/cutter (2,500 cpm) through the instrument port (adjacent to the surgeon's dominant hand, i.e., the 10 o'clock port for a right-handed surgeon) and perform a partial core vitrectomy.

3.9.1. Then, completely remove the vitreous from the retinal surface in the region that will receive the transplant (this is important for the success of the procedure) by detaching the vitreal face from the retina.

3.9.2. Place the vitrectomy probe over the optic nerve head with the port facing away from the retinal surface and apply higher vacuum to start the vitreal face detachment.

3.10. Prepare triamcinolone crystals for intraocular use. If the triamcinolone suspension is not specifically for intravitreal use, wash the crystals and then re-suspend in BSS.

3.10.1. Initially, filter the suspension with the aid of a sterile 0.22 µm pore syringe filter with PES membrane (attached to a 1 mL syringe) to trap the crystals.

3.10.2. Then wash the trapped triamcinolone crystals by aspirating BSS in the 1mL syringe and flushing through the filter (the crystals remain trapped in the filter). This removes the preservatives in the solution. Repeat the washing 3 times after which the crystals are re-suspended in 1 mL BSS.

3.11. Introduce the needle of the syringe holding the triamcinolone through the instrument port. Be careful not to touch the lens by watching the tip of the needle through the pupil while introducing the needle through the instrument port. Inject 0.25 to 0.5 mL of crystal suspension. Then insert the vitrectomy probe through the instrument port and advance it close to the optic

nerve head with the port away from the retinal surface and use high vacuum to help detach the vitreal face from the retina.

NOTE: The triamcinolone crystals stick to and thus highlight the remaining vitreous. Carefully remove any vitreous above and next to the site of planned organoid implantation (e.g., the central tapetal fundus close to the *area centralis* region).

3.12. Create a small focal retinal detachment bleb at the desired implantation site using a subretinal injector, e.g., 23 G subretinal injector with an extendable 41 G cannula attached to a sterile 250 µL gas tight Luer lock syringe filled with sterile BSS.

3.12.1. Prior to creating the subretinal bleb, reduce the infusion pressure to 10 mmHg to facilitate bleb formation.

3.12.2. Insert the injector through the instrument port and advance it toward the retinal surface. Extrude the cannula tip and gently press it on to the retinal surface. Ask the assistant to give a slight quick push on the syringe plunger to start the retinal detachment that reduces the injection pressure to permit a slow increase of the retinal detachment until the desired size is achieved (approximately 100 to 200 µL of BSS is used).

3.13. If the retinotomy created is at a suitable position, which avoids cutting the retina between the implantation site and the optic nerve head to prevent the sectioning of the nerve fiber layer derived from the implantation site and to avoid major retinal blood vessels (this is also determined by the study design, in our case the central retina was chosen), then, slightly enlarge it using the 41 G cannula of the injector, aiming to facilitate the introduction of retinal scissors.

3.14. Remove the injector from the eye and remove the 10 o'clock scleral port. Enlarge the sclerotomy at this site using a straight 2.85 mm slit knife/keratome oriented toward the optic nerve to avoid touching the lens. Maintain the infusion pressure at 10–15 mmHg.

3.15. Extend the retinotomy using vitreoretinal vertical 80° scissors with squeeze handle, avoiding cutting retinal vessels to prevent retinal hemorrhage and be sure to cut the retina at the edge of the bleb away from the optic nerve head to avoid cutting nerve fibers leading from the retina in the transplant area to the optic nerve. The retinotomy should be of sufficient width to receive the organoids.

3.16. Insert the pre-loaded glass capillary containing the organoids (see step 2.10) through the enlarged sclerotomy and advance it toward the retinotomy site under direct visualization.

3.16.1. Use the tip of the glass capillary to slightly open the retinotomy to access the opening into the subretinal bleb.

3.16.2. Ask the assistant to slowly press the plunger of the injector, while watching through the microscope, injecting the organoids into the subretinal bleb. BSS should precede the organoids and flush the retinotomy open.

3.16.3. Gently push the organoids within the bleb with the tip of the glass capillary if they are at the edge of the retinotomy.

3.17. Hold the glass capillary at the retinotomy for a few seconds to try and close the retinotomy and prevent the organoids from escaping into the vitreous.

3.18. Very slowly remove the glass capillary from the eye avoiding any sudden release of fluid from the eye to avoid fluid movement within the eye that might expel the organoids from the subretinal bleb.

3.19. Slowly increase the infusion pressure to 20–30 mmHg to help prevent intraocular hemorrhage taking care that the infusion fluid does not wash directly onto the bleb. This is performed by pressing on the upward arrow buttons present on the front panel of the vitrectomy machine.

3.20. Close the sclerotomy using the pre-placed suture in a cruciate pattern (6–0 or 7–0 polyglactin). Add additional simple interrupted sutures as needed to seal the sclerotomy.

3.21. Ask the assistant to remove the infusion port and let the surgeon quickly tie the pre-placed suture to seal the sclerotomy and prevent loss of intraocular pressure.

3.22. Close the conjunctival incision (peritomy) using 6–0 or 7–0 polyglactin in a simple continuous pattern.

3.23. Image the fundus (e.g., using a RetCam II video fundus camera, Clarity, or similar) and record the immediate position of the organoids within the subretinal space.

3.24. Re-prep the ocular surface after imaging using 0.2% povidone iodine solution with the aid of cotton tip applicators and cotton balls. Remove the three stay sutures and the lid speculum.

3.25. Close the lateral canthotomy with 6-0 polyglactin suture. Place a single buried suture followed by a figure of 8 skin sutures to reform the lateral canthus and then use simple interrupted skin sutures to close the rest of the wound.

3.26. After the surgical procedure is completed, give a subconjunctival injection of a steroid and antibiotic combination (2 mg methylprednisolone acetate, 0.1 mg dexamethasone, and 1 mg gentamicin). Place an ophthalmic lubricant (artificial tears) on the cornea.

3.27. Recover the animal from anesthesia and monitor closely during recovery. Provide post-operative analgesia as needed and monitor closely for any discomfort.

4. Post-implantation procedures, post-operative treatment, and assessment

4.1. Continue to treat with oral immunosuppressive medications (1 mg/kg prednisolone and 2 mg/kg cyclosporine orally twice a day) to help control inflammation and rejection of the organoids. Provide systemic antibiotic coverage (e.g., oral doxycycline 5 mg/kg twice daily – this antibiotic was selected because of the risk of opportunist mycoplasmal infection in immunosuppressed animals).

4.2. Perform regular ophthalmic examinations to monitor for inflammation or the development of adverse events. Monitor the retinal bleb for flattening, which occurs over the first few days following surgery despite the large retinotomy required for organoids to be injected into the subretinal space. Record fundus images at each examination to record the position and appearance of the transplanted organoids.

4.3. Image the animals under general anesthesia by confocal scanning laser ophthalmoscopy (cSLO) and spectral domain – optical coherence tomography (SD-OCT)[5,31] during the monitoring period and prior to the termination of the experiment.

4.4. Humanely euthanize the animals at the termination of the experiment using an AVMA approved method, e.g., intravenous administration of 85 mg/kg of pentobarbital. After sedation, place an intravenous catheter for administration of the pentobarbital to minimize stress. Confirm the death by cessation of heartbeat and make an incision into the thorax to create a pneumothorax.

4.5. Remove the eyes via a standard transconjunctival approach and fix as required. For immunohistochemistry (IHC), immediately immerse the eyes in 4% paraformaldehyde solution after 0.3 mL of the fixative solution is injected into the posterior segment through 3 slits made through pars plana with a 11 Parker blade (~3-4 mm from the limbus).

4.6. Dissect the eyecups after 3.5 h of fixation at 4 °C. Remove residual vitreous making sure to preserve the retina and organoids intact. Place back in fixative for an additional 30 min at 4 °C then rinse the eyecups 3 times each for 10 min in PBS. Transfer the eyecup to 15% sucrose for 2 h, then to 30% sucrose for a further 2 h.

4.7. Rinse the eyecup twice with PBS and embed in OCT medium (optimal cutting temperature compound) and flash freeze then store at -80 °C until sectioning for histology and immunochemistry.

4.8. Select antibodies for IHC based on the study protocol and aims.

REPRESENTATIVE RESULTS:

This procedure enables the successful and reproducible implantation of hPSC-derived retinal organoids in the subretinal space of a large eye animal model (demonstrated here using 2

examples: wild-type cats with healthy photoreceptors (PRs) and *Crx*$^{Rdy/+}$ cats with degenerating PRs and retina). Using the steps indicated in **Figure 1** prepare and load the hPSC-derived retinal organoids into the borosilicate glass cannula of the injection device so that the organoids are not damaged. This can be confirmed by direct visualization during the loading of the organoids (step 2.10) and during the surgery (step 3.12.2 and step 3.16) (**Figure 2A,B**) as well as by fundus imaging at the end of surgery (step 3.23, **Figure 2C**). The presence of the organoids in the subretinal space using this technique is confirmed post-operatively by ophthalmic examination and fundus imaging (**Figure 3A**), which records the position and appearance of the organoids. Knowing the position of the transplant is very important when processing the globes for frozen histology and immunohistochemistry and substantially reduces workload, as sectioning a large eye at 12–14 µm (thickness of a cryosection) takes time. Prior to euthanasia, confocal scanning laser ophthalmoscopy (cSLO) and spectral domain – optical coherence tomography (SD-OCT) imaging are also performed to assess the position of the organoids in the subretinal space (**Figure 3A-D**). These techniques demonstrate the persistence of retinal organoids in the subretinal space (between the neural retina and RPE) of the recipient eye (**Figure 3E**). Following euthanasia (done humanely following AVMA recommendations) the histology and immunohistochemistry (IHC) is routinely performed (see details in previously published paper[31]). The histology and IHC demonstrate the survival of xenogeneic grafts (hPSC-derived retinal organoids) in the subretinal space of a large eye when the animals were immunosuppressed (as previously described[31]), see **Figure 4**.

FIGURE LEGENDS:

Figure 1: Schematic of the steps for organoids preparation prior to the implantation.

Figure 2: Surgical subretinal implantations of organoids. (**A**) Direct visualization of organoids being delivered into the subretinal space through a glass cannula without being damaged, (**B**) Direct visualization of organoids in the subretinal bleb, (**C**) Wide-angle fundus color image of the subretinally implanted organoids immediately after surgery. The bleb edges are indicated by the black arrowheads and the retinotomy site by the black stars.

Figure 3: Post-operative assessment of subretinally implanted organoids 3 months post-implantation in a *Crx*$^{Rdy/+}$ cat. (**A**) Fundus color image of the subretinally implanted organoids, (**B**) cSLO fundus image of the subretinally implanted organoids, (**C**) 3D volume scan reconstruction of the area containing the organoids, (**D**) cSLO image of the area containing subretinal organoids, (**E**) SD-OCT high-resolution, cross-section image of the subretinally implanted organoids. The retinotomy site is indicated by the black stars.

Figure 4: Human retinal organoid-derived photoreceptor sheets (PR marker RCVRN) in subretinal space of *Crx* $^{Rdy/+}$ cat, 3 months after grafting. *Synaptic boutons (hSYP=Synaptophysin) in cat inner nuclear layer (INL).

DISCUSSION:

Implantation of hPSC-derived retinal tissue (retinal organoids) into the subretinal space is a promising experimental approach for restoring vision for late-stage retinal degenerative diseases caused by PR cell death (profound or terminal blindness). The presented approach builds on an earlier developed and successfully tested experimental therapy based on subretinal grafting of a piece of human fetal retinal tissue[23-25]. It presents the use of an alternative, replenishable and ethically acceptable retinal tissue source derived from hPSCs. Demonstrating surgical feasibility and ocular safety of therapy in large eye animal models[51,55] is needed for advancement of this promising approach toward clinical applications. This manuscript provides a detailed method for subretinal implantation of hPSC-3D retinal tissue (retinal organoids) in a large animal model with both normal and degenerating retina (model of *Crx*$^{Rdy/+}$ cats model of LCA). Though procedures for introducing a flat piece of human retina and also RPE patches have been developed[43-45], transplantation of larger 3D grafts (needed to restore vision in conditions with advanced RD) have not been investigated. The protocol described here in detail is for a transplantation procedure for subretinal delivery of the whole retinal organoids (rather than organoid rims[33,41]) also carrying some RPE as a potentially better way to introducing intact retinal sheets with PRs, to increase graft survival and improve the sheet preservation. Note that different portions of this protocol are well established. For example, vitrectomy is widely used by vitreoretinal surgeons during retinal reattachment surgery[56-60]. Subretinal injections are becoming more commonly used, for example, in gene augmentation therapy[3,7,61-64]. There are limited descriptions of creation of an adequate retinotomy and the injection of relatively large organoids into the subretinal space.

The critical steps include careful positioning and performance of the sclerotomy to avoid the lens, complete removal of vitreous cortex from the retinal surface over the transplant site, controlled formation of the subretinal bleb, generating retinotomy of the optimal size to accommodate the width of the transplantation cannula, maintaining the defined infusion pressure during different steps, and cannula withdrawal. Choosing the right transplantation cannula with optimized inner and outer diameter (ID and OD) and length, controlling intraocular bleeding, sterility of the whole procedure from organoids to surgical room and instruments, and the duration of surgery (30–45 min/animal) to ensure optimal results. The authors find that the best results are obtained with 23 G vitrectomy due to the thickness/viscosity of the cat vitreous, creating a bleb with an injector with a 41 G cannula, and then, extending the retinotomy at the edge of the bleb using 80° angle retinal scissors. Other important factors include extending the sclerotomy using a 2.85 mm keratome to fit the borosilicate glass cannula (outer diameter OD 1.52 mm; inner diameter, ID 1.12 mm; and length 10.16 cm) and permitting implantation of larger organoids in a large eye with axial length about 20.5 mm (20.91 mm ± 0.53 mm)[55]. The use of a glass capillary was the current best available for loading and delivering descent size organoids without damaging them during the implantation process. It was found that reducing the infusion pressure from 20–30 mmHg during the vitrectomy to 10 mmHg during the bleb formation step is optimal for generating retinal detachments, needed for creating space for retinal organoids. In addition, viability of hPSC-3D retinal tissue (organoids) during long-distance shipment and choosing the optimized immunosuppression regimen for maintenance of xenogeneic human grafts are critical as recently reported[31,54].

The authors found that due to the highly reflective cat tapetum endo-illumination was not required to perform the surgery. Transplantation in an atapetal species (e.g., pig, nonhuman primate would require a 3-port vitrectomy that includes endo-illumination). Tamponade (e.g., with perfluorocarbon followed by silicone oil) as performed in routine retinal detachment surgery was not performed because pressure on the bleb would risk extruding the organoids into the vitreous. Future developments could include the use of materials currently being investigated for sealing retinal holes. This could be used to prevent any post-implantation loss of organoids into the vitreous. Additionally, Triescence, which is similar to Kenalog-40 but contains preservative-free triamcinolone, could be used as an alternative to visualize the vitreous.

The smaller globe size in younger animals (e.g., 1–2 months) presented more surgical challenges compared to the large eye (adult animals). Nevertheless, using the method described here, it is possible to deliver the subretinal grafts. In the *Crx*$^{Rdy/+}$ LCA model, larger retinal blebs were found to not always reattach. Keeping the bleb to the minimum size needed to allow the retinal organoids to be injected, reduced this complication. Transplanting sooner in RD retina (before the complete loss of PRs when neural retina becomes markedly thinned) is another guideline, in line with the earlier work with human fetal retina grafts[20]. Another note – the detailed technique does not depend on placing the retinal sheet in correct orientation because the whole retinal organoids are transplanted. With development of flat hESC-retinal sheets, this will be important. However, it is not the focus of this protocol, which is aimed at delivering intact hESC-retinal tissue sheets and optimizing the subretinal preservation of PR sheets. Once the technique was developed, it was reproducible in both normal and RD retina.

There are many potential complications to this surgical procedure. Only those skilled in vitreoretinal surgery (e.g., trained veterinary ophthalmologists or vitreoretinal surgeons familiar with the species differences in the cat eye compared to human eye) should undertake the procedure. Possible complications include lens touch during trocar placement, scleral bleeding during sclerotomy enlargement, and subretinal or retinal hemorrhages. Other complications such as the host immune response to xenogeneic human organoid grafts leading to destruction of the graft over time or endophthalmitis are possible; the use of oral immunosuppressant and antibiotic medications help prevent these from occurring. It is also important to note there is a difference between implantation in wildtype and *Crx*$^{Rdy/+}$ cats. When there is advanced retinal degeneration, the retinal bleb tends to spread wider than in the wildtype, and, in some instances, this can prevent complete retinal reattachment after the surgery. The technique presented here is applicable for implantation of organoids in eyes of large animal models of IRDs. Further refinement would be needed once the transplantation is ready for translation to the clinic.

Based on the authors' experience with performing complex vitreoretinal surgical procedures in large eye models, the technique presented in this manuscript should be applicable to other large animal models (with the inclusion of endo-illumination in atapetal species) that are used for translating vitreoretinal surgical techniques to the clinic[43-45].

ACKNOWLEDGMENTS:

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DISCLOSURES:

Ratnesh K. Singh, Ph.D., Francois Binette, Ph.D., and Igor O. Nasonkin Ph.D. are employees of Lineage Cell Therapeutics, Inc. The authors declare no conflict of interest.

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Figure 1

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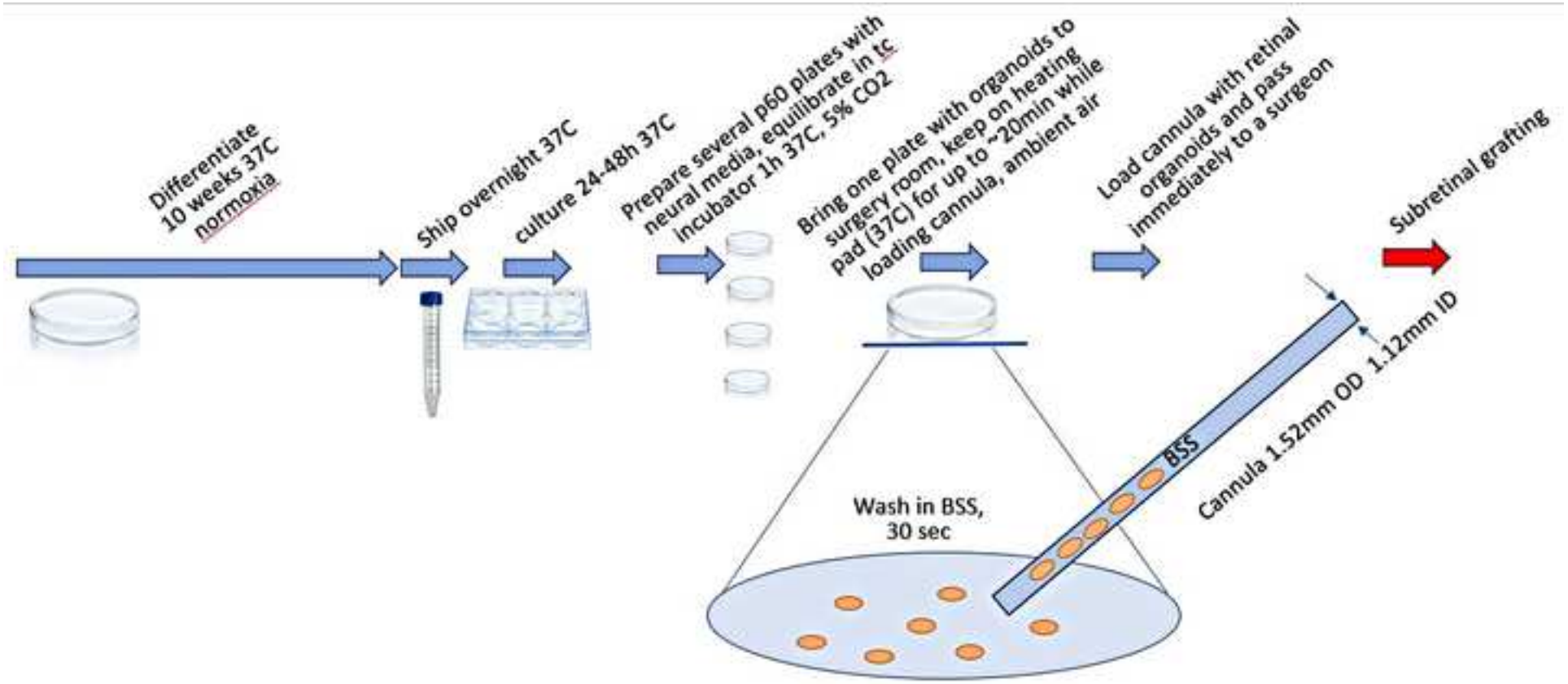

Figure 2

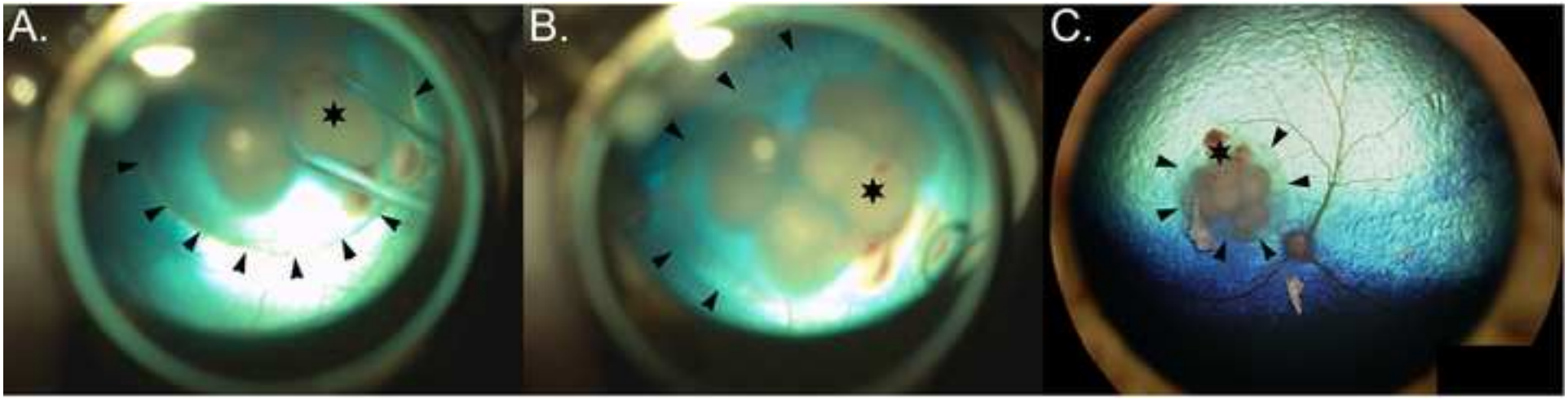

Figure 3

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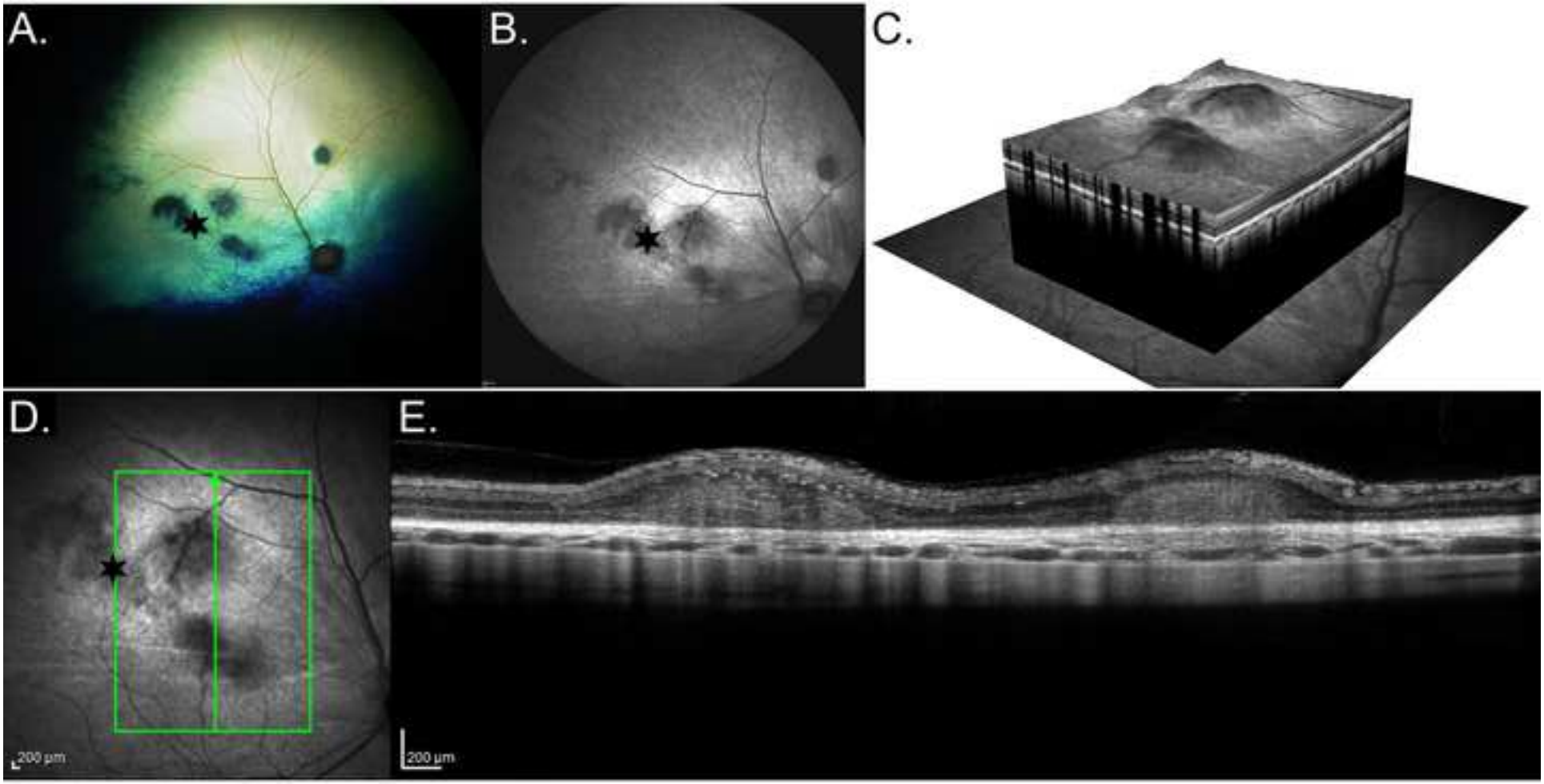

Figure 4

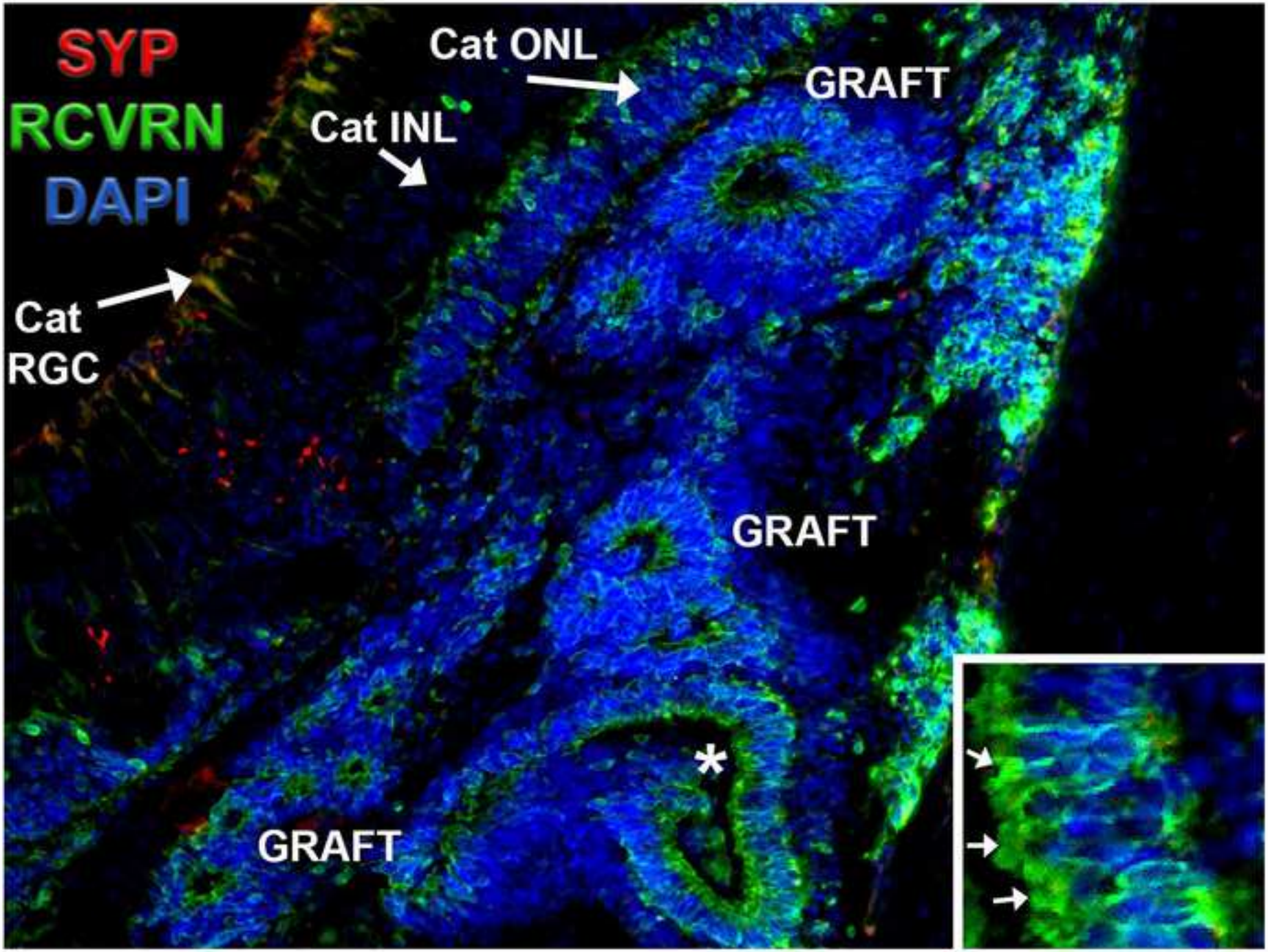

Table of Materials

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Name of Material/Equipment	Company	Catalog Number
0.22 µm pore syringe filter with PES membrane	Cameo	NA
23G subretinal injector with extendable 41 G cannula	DORC	1270.EXT
250 µL hamilton gas tight luer lock syringe	Hamilton	NA
6-0 Silk suture	Ethicon	707G
6-0/7-0 polyglactin suture	Ethicon	J570G
Acepromazine maleate 500mg/5mL (Aceproject)	Henry Schein Animal Health	NA
Buprenorphine 0.3 mg/mL	Par Pharmaceutical	NA
cSLO + SD-OCT	Heidelberg Engineering	Spectralis HRA+ OCT
Cyclosporine	Novartis	NA
Dexamethasone 2mg/mL (Azium)	Vetone	NA
Doxycyline 25mg/5mL	Cipla	NA
Fatal Plus solution (pentobarnital solution)	Vortech	NA
Gentamicin 20mg/2mL	Hospira	NA
Glass capillary (Thin-Wall Single-Barrel Standard Borosilicate (Sch	World Precision Instruments	TW150-4
Methylprednisolone actetate 40 mg/mL	Pfizer	NA
Microscope	Zeiss	NA
OCT medium (Tissue-Tek O.C.T. Compound)	Sakura	4583
Olympic Vac-Pac Size 23	Natus	NA
Paraformaldehyde 16% solution	EMS	15719
Phenylephrine Hydrochloride 10% Ophthalmic Solution	Akorn	NA
Prednisolone 15mg/5mL	Akorn	NA
Propofol 5000mg/50mL (10 mg/mL) (PropoFlo28)	Zoetis	NA
RetCam II video fundus camera	Clarity Medical Systems	NA
Triamcinolone 400mg/10 mL (Kenalog-40)	Bristol -Myers Squibb Company	NA
Tropicamide 1% ophthalmic solution	Akorn	NA
Vitrectomy 23G port	Alcon	Accurus systems
Vitrectomy machine	Alcon	Accurus systems
Vitreo-retinal vertical 80° scissors with squeeze handle	Frimen	FT170206T

Comments/Description
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Managing Editor Dr. Vineeta Bajaj
JoVE

August 3rd, 2020

Re: Review Editor JoVE
JoVE61683R1 "A Method for Subretinal Transplantation of Human Embryonic Stem Cell-Derived Retinal Tissue Transplantation in a Feline Large Animal Model"

Dear Dr Bajaj

We are returning our corrected manuscript for JoVE61683R1.

We wanted to clarify a couple of things.

Editorial comment on line 202-203. 2.1 of method. We do not understand the comment made there. Reviewer 1, Comment 3 was referring to the vitrectomy technique. We have added references to the discussion to cover that point. At the point the comment is inserted (Step 2.1) we have cited references 31 and 54 for the methods of deriving organoids. Can you clarify what changes you would like.

Video. We have supplied a version of the video at normal speed and with no text. This runs for 31 minutes. We are assuming that this is too long and we will need to miss out some details. Before definitely linking text to specific video portions we feel that this needs to be addressed (we yet have provided a word document indicating at which minute/second the highlighted steps in the text are present in the video). Can you let us know if this video will work or whether it should be broken up into short video segments covering the most critical portions of the surgery.

Please feel free to contact us if you would require more information. Thank you for your time and consideration.

Sincerely,

Simon Petersen-Jones for the authors.

VIDEO: Step-Video timings

Subretinal Transplantation of Human Embryonic Stem Cell-Derived Retinal Tissue in a Feline Large Animal Model

Step	Time	Step description highlighted in the manuscript
3.1	00:07 00:20 00:30	Perform a 0.5 to 1 cm lateral canthotomy with Stevens tenotomy scissors. Place an appropriately sized Barraquer eyelid speculum to keep the eyelids open. Ensure that the surgical assistant irrigates the cornea regularly with BSS for the duration of the procedure.
3.2.	01:18 01:35	Use 0.5 Castroviejo corneal tying forceps and a small mosquito hemostat to gently grasp the bulbar conjunctiva next to the limbus. Place a stays suture of 6-0 silk suture in the conjunctiva immediately adjacent to the limbus at the 8 o'clock position to hold the eye in primary gaze and retract the third eyelid. Leave the sutures long and clamped the ends with small mosquito hemostats to help with their manipulation.
3.3.	02:17	Place another suture at the 12 o'clock position at the limbus partially through the thickness of the limbus taking care not to penetrate the eye. Knot this suture loosely and cut the ends short. This provides a robust anchor suture which is used during the surgery as a "handle" to rotate the eye to allow the surgeon access to different parts of the globe during different stages of the procedure.
3.2.	03:40	Place a stay suture of 6-0 silk suture in the conjunctiva immediately adjacent to the limbus at the 4 o'clock positions to hold the eye in primary gaze.
3.4.	04:43	Reflect the bulbar conjunctiva between 10 – 2 o'clock (a perimetry). Using tenotomy scissors incise the conjunctiva 2-3 mm from the limbus, undermine it and clear tenon's capsule to expose the sclera at the sites for the 2 and 10 o'clock vitrectomy ports which will be placed about to 3-5 mm from the limbus depending of the age of the animal.
3.5	05:18 05:40 05:52	Identify sites for sclerotomy following the reflection of the conjunctiva and tenon's capsule using calipers. Choose sclerotomy sites (at 2 and 10 o'clock aiming to pass through pars plana), to avoid the major scleral vessels that can be prominent in the cat. Use surgical cellulose spears for hemostasis and to clear any blood. Use wet-field cautery on the sclera at the planned sclerotomy sites to reduce scleral bleeding, planning for a ~ 3 mm region at the instrument port (10 o'clock port for a right-handed surgeon) as this will be enlarged following the vitrectomy to allow the implantation cannula to be introduced.
3.6.	06:45	Pre-place cruciate pattern sutures (6-0 or 7-0 polyglactin suture) without tying the knot at the site of proposed sclerotomies before the vitrectomy ports are placed.
3.7.	11:13	Once the sutures are placed, ask the assistant to help position the globe by holding the 12 o'clock stay sutures by tying or using Bishop-Harmon forceps.

	11:25	Let the surgeon stabilize the globe as well, using a 0.12 mm Castroviejo forceps to hold the tissue next to the sclerotomy site.
3.7.1.	11:26	Introduce a 23 G vitrectomy port using a trocar through the sclera at both the 2 o'clock and 10 o'clock positions directed at an angle towards the optic nerve to avoid contacting the lens.
3.7.2.	12:13	Check that the irrigation port is in the vitreous by gently pushing on the port using a tying forceps so that the tip can be visualized in the vitreous.
3.8.	13:21	Let the assistant hold a Machemer magnifying irrigating vitrectomy lens onto the cornea to allow visualization of the posterior segment of the eye during the next stages.
3.9.	13:25	Insert the 23 G vitrectomy probe/cutter (2,500 cpm) through the instrument port (adjacent to the surgeon's dominant hand, i.e., the 10 o'clock port for a right-handed surgeon) and perform a partial core vitrectomy.
3.11.	14:45 14:50 15:08	Introduce the needle of the syringe holding the triamcinolone through the instrument port. Inject 0.25 to 0.5 mL of crystal suspension. Then insert the vitrectomy probe through the instrument port and advance it close to the optic nerve head with the port away from the retinal surface and use high vacuum to help detach the vitreal face from the retina.
3.12.2.	16:08	Insert the injector through the instrument port and advance it towards the retinal surface. Extrude the cannula tip and gently press it on to the retinal surface. Ask the assistant to give a slight quick push on the syringe plunger to start the retinal detachment then reduces the injection pressure to permit a slow increase of the retinal detachment until the desired size is achieved (approximately 100 to 200 µL of BSS is used).
3.13.	16:30	Slightly enlarge it using the 41 G cannula of the injector, aiming to facilitate the introduction of retinal scissors.
3.14.	16:39 16:57	Remove the 10 o'clock scleral port. Enlarge the sclerotomy at this site using a straight 2.85 mm slit knife/keratome oriented towards the optic nerve to avoid touching the lens.
3.15.	17:14	Extend the retinotomy using vitreoretinal vertical 80° scissors with squeeze handle, avoiding cutting retinal vessels to prevent retinal hemorrhage and be sure to cut the retina at the edge of the bleb away from the optic nerve head to avoid cutting nerve fibers leading from the retina in the transplant area to the optic nerve.
3.16.	17:43 17:58	Insert the preloaded glass capillary containing the organoids (step 2) through the enlarged sclerotomy and advance it towards the retinotomy site under direct visualization.
3.16.1.	18:05	Use the tip of the glass capillary to slightly open the retinotomy to access the opening into the subretinal bleb.
3.16.2.	18:06	Ask the assistant to slowly press the plunger of the injector while watching through the microscope injecting the organoids into the subretinal bleb. BSS should precede the organoids and flushes the retinotomy open.

3.16.3.	18:18	Gently push the organoids within the bleb with the tip of the glass capillary if they are at the edge of the retinotomy.
3.17.	18:20	Hold the glass capillary at the retinotomy for a few seconds to try and close the retinotomy and prevent the organoids from escaping into the vitreous.
3.18.	18:30	Very slowly remove the glass capillary from the eye avoiding any sudden release of fluid from the eye to avoid fluid movement within the eye that might expel the organoids from the subretinal bleb.
3.20.	18:53	Close the sclerotomy using the preplaced suture in a cruciate pattern (6-0 or 7-0 polyglactin).
3.21.	20:52	Ask the assistant to remove the infusion port and let the surgeon quickly tie the pre-placed suture to seal the sclerotomy and prevent loss of intraocular pressure.
3.22.	22:15	Close the conjunctival incision (peritomy) using 6-0 or 7-0 polyglactin in a simple continuous pattern.
3.25.	26:25 28:40	Close the lateral canthotomy with 6-0 polyglactin suture. Place a single buried suture followed by a figure of 8 skin suture to reform the lateral canthus then use simple interrupted skin sutures to close the rest of the wound.
3.26.	31:30	After the surgical procedure is completed, give a subconjunctival injection of a steroid and antibiotic combination (2 mg methylprednisolone acetate, 0.1 mg dexamethasone and 1 mg gentamicin.

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Dr. Occelli did her Veterinary training in France and graduated in 2005. She pursued further education in ophthalmology in France, Canada and United States as well as in anesthesia in England. She completed her PhD within the Comparative medicine and integrative biology at Michigan State University. During her PhD she worked on further characterizing and understanding the dominant negative underlying mechanism in the *CRX*-LCA feline large animal model.

LUIS FELIPE LIMA POMPEO MARINHO

Luis Felipe Lima Pompeo Marinho DVM, MSc graduated at University of Marília - Brazil in 2002. After that, he attended the 4 year combined ophthalmology training and Master degree at São Paulo State University. He then founded a veterinary ophthalmology private practice clinic in his hometown Santos –Brazil where he worked for 8 years.
Dr. Marinho completed his 2.5 years postdoctoral fellowship at the Retinal Disease Studies Facility (University of Pennsylvania) in 2017. In 2018, he joined the Simon Petersen-Jones lab as a Researcher at Michigan State University researching inherited retinal diseases and gene therapy in large animal models.

Igor O. Nasonkin Ph.D. is Director of R&D at Lineage Cell Therapeutics, Inc. (former BioTime, Inc.) and specializes in therapies of neurodegenerative diseases, including cell therapies and tissue replacement in degenerating retina.

Dr. Nasonkin received B.Sc. degree in Biochemistry (2nd major: molecular genetics) at St.Petersburg University in 1992. He then joined M.Sc. program in Biochemistry at the U.British Columbia (1995-97), and after that -Ph.D. program in human genetics at the U.Michigan -Ann Arbor (1997-2002). He then completed postdoctoral training at MIT (Whitehead Institute, Cambridge), Johns Hopkins (Baltimore) and Bresagen, Ltd, and then became a senior fellow at National Eye Institute with Dr. Anand Swaroop. He was a faculty in ophthalmology at the U.Pittsburgh until joining BioTime, Inc. (now LCTX) to lead preclinical retinal restoration work using retinal tissue derived from human pluripotent stem cells.

Dr. Binette has B.Sc. (biochemistry, 1995) and PhD (biology, 1990) from Université Laval in Québec City, followed with post-doctoral training at the Sanford-Burnham institute in La Jolla, and Harvard Medical School in Boston. He has over 25 years of experience in regenerative medicine therapy development. During his first industry appointment at Genzyme Tissue Repair in Cambridge, he helped pioneer Carticel™ for cartilage repair, the first FDA BLA-approved cell therapy product for human use. He then led R&D for Biosyntech, a startup biomaterials company in Montreal applying its proprietary platform for various tissue engineering and drug delivery applications. His systematic approach to demonstrating proof of principle in various indications brought numerous corporate partnership deals helping Biosyntech through a public offering. Dr. Binette then joined the DePuy Franchise of Johnson and Johnson, the second largest orthopedic business worldwide. Dr. Binette led several innovative regenerative medicine combination product development initiatives from bench top to approved clinical trials in US and Europe. Following JNJ, Dr. Binette joined the Spinal and Biologics business unit of Medtronic, the world's largest medical device company, developing biologics, active devices and combination products for interventional spine procedures for back pain. He is currently Sr. Vice President and Global Head of Product Development at Lineage Cell Therapeutics (former BioTime).

Ratnesh Singh PhD, is the Senior scientist at the Lineage Cell Therapeutics Inc where he is involved in developing allogeneic cell therapy based product and driving preclinical research to clinical trial.

Dr. Singh did his postdoctoral research from West Virginia University (WVU) and at University of Pittsburgh (U Pitt). At WVU, his work was focused on understanding biochemical mechanism behind retinal degenerative diseases. At U Pitt he was extensively working on differentiating human pluripotent stem cells into three-dimensional retinal organoid. During his postdoctoral research he received prestigious knight templar grant for his work on understanding lipid modifications of proteins needed for light perception.

Dr. Simon Petersen-Jones received his veterinary training at the Royal Veterinary College, University of London. He then completed a clinical veterinary ophthalmology residency at the same institution and a research doctorate concurrently. He was Head of the Clinical Ophthalmology Service at the Royal (Dick) School of Veterinary Studies, University of Edinburgh for 6 years. He then moved to University of Cambridge under a Wellcome Trust Veterinary Career Development Fellowship. While at Cambridge he also completed a PhD program. He was appointed a Diplomate of the European College of Veterinary Ophthalmologists, he was also awarded a Pfizer Award for research contributions and received the British Small Animal Veterinary Association Simon Award for contributions to veterinary surgery. He moved to Michigan State University where he has spent the last 20 years with clinical, research and teaching commitments. He is now the Myers-Dunlap Endowed Chair in Canine Health. His research interests include characterization of spontaneous hereditary eye disease in dogs and cats and development of potentially translatable therapy for treatment of retinal degenerative disease. He has been involved in preclinical testing of gene therapy for Leber Congenital Amaurosis and Retinitis Pigmentosa. At Michigan State he was also awarded the Pfizer Research Award for his research contributions.