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Transpupillary-Guided Trans-Scleral Transplantation of Subretinal Grafts in a Retinal Degeneration Mouse Model

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Abstract

Transplantation of photoreceptor cells and retinal pigment epithelial (RPE) cells provide a potential therapy for retinal degeneration diseases. Subretinal transplantation of therapeutic donor cells into mouse recipients is challenging due to the limited surgical space allowed by the small volume of the mouse eye. We developed a trans-scleral surgical transplantation platform with direct transpupillary vision guidance to facilitate the subretinal delivery of exogenous cells in mouse recipients. The platform was tested using retinal cell suspensions and three-dimensional retinal sheets collected from rod-rich *Rho::EGFP* mice and cone-rich *OPN1LW-EGFP;NRL*$^{-/-}$ mice, respectively. Live/dead assay showed low cell mortality for both forms of donor cells. Retinal grafts were successfully delivered into the subretinal space of a mouse model of retinal degeneration, *Rd1/NS*, with minimum surgical complications as detected by multimodal confocal scanning laser ophthalmoscope (cSLO) imaging. Two months post-transplantation, histological staining demonstrated evidence of advanced maturation of the retinal grafts into 'adult' rods and cones (by robust Rho::EGFP, S-opsin, and OPN1LW:EGFP expression, respectively) in the subretinal space. Here, we provide a surgical platform that can enable highly accurate subretinal delivery with a low rate of complications in mouse recipients. This technique offers precision and relative ease of skill acquisition. Furthermore, the technique could be used not only for studies of subretinal cell transplantation but also for other intraocular therapeutic studies including gene therapies.

Introduction

Transplantation of photoreceptor and retinal pigmented epithelial (RPE) cells provides potential therapy for retinal degenerative diseases such as age-related macular degeneration (AMD), Stargardt disease, and retinitis

pigmentosa (RP)[1,2,3,4,5,6,7]. To replenish or replace the diseased photoreceptors and RPE cells in degenerated retinae, the subretinal space is particularly well suited as a transplantation target given the laminar anatomy of host photoreceptors and RPE cells. While surgical procedures of sub-retinal transplantation of RPE cells are well-established in large animals[8,9,10] and clinical trials[11,12,13], challenges facing photoreceptor transplantation research include the scarcity of transgenic large animal models and the limited understanding of in-depth synaptogenesis mechanisms involved in neuronal transplantation, among other concerns. Genetically modified murine models, with various types of retinal degeneration mutants, provide useful tools for studying molecular mechanisms in the context of transplantation and steering the development of effective cell replacement therapies at the preclinical stage[14,15,16,17,18].

Unlike the relatively large eye and small crystalline lens in large animals (e.g., pig, monkey), the small size and large crystalline lens of mouse eyes make them difficult surgical targets, especially for subretinal transplantation in which physical space constraints and limited direct visualization are the core challenges.

Current approaches can be classified into three major types based on the injection route. First, in the case of the trans-corneal approach, the needle is passed through the cornea into the vitreous cavity and then into the subretinal space[19,20]. Successful subretinal delivery can be achieved using this method but the damage to anterior segment structures (i.e., cornea, iris, lens) is a major risk that may severely impede downstream *in vivo* analysis. Second, in the case of the trans-vitreous approach, the needle enters the vitreous cavity through the pars plana and then into the subretinal space[21]. This approach is widely used in humans and large animals. However, there is a potential risk of lens damage in rodents as the lens occupies a larger relative volume of the vitreous cavity. Notably, both trans-corneal and trans-vitreous protocols require penetration of the neural retina to arrive at the subretinal space, which causes damage to the host retina and increases the risk of donor cell reflux through the penetration hole. Third, in the case of the trans-scleral approach[22,23], the needle penetrates through the scleral-choroid-RPE complex and directly enters the subretinal space. This approach reduces the potential trauma of anterior segment structures and the vitreous cavity. However, without direct visualization of the host fundus, surgical failures caused by trans-retinal punctures, RPE detachment, and choroid hemorrhage are commonly detected.

Here, we developed a trans-scleral surgical platform with direct transpupillary vision guidance for subretinal transplantation in mouse recipients. Validation of the viability of donor retinal sheets and cell suspensions before transplantation was performed. Successful delivery of the donor cells into the subretinal space of a retina degeneration mouse model was confirmed. Only rare surgical complications were detected. In addition, transplanted photoreceptors in the retinal grafts survived and showed evidence of advanced maturation into "adult" rods and cones two months post-transplantation.

Protocol

All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All procedures were

approved by the Johns Hopkins University Animal Care and Use Committee (approval M021M459).

1. Animals

1. Use *Rho::EGFP* mice (aged P3-P6) as donors of retinal cell suspensions.
   NOTE: This strain was a kind gift from Dr. T. Wensel, Baylor College of Medicine.
2. Use *OPN1LW-EGFP/NRL*$^{-/-}$ mice[14] (aged P3) as donors of retinal sheets.
3. Use adult retinal degeneration *Rd1/NS* mice with immune deficiency (either sex) as recipients.
4. House all mice in cages under a 12:12 h light-dark cycle with access to water and food as needed.

2. Collect neural retina (Figure 1)

NOTE: All the following steps were performed under sterile conditions. Supplier information of the research tools and products is provided in the **Table of Materials**.

1. Donor mice preparation: Euthanize donor mice with an overdose of carbon dioxide and confirm euthanasia with cervical dislocation.
2. Isolate mice eyeballs. To do so follow the steps below.
   1. Carefully open pups' eyelids using micro scissors to expose the eyeball.
   2. Use smooth forceps to grab the optic nerve and pull out the eyeball with a pair of forceps.
3. Isolate neural retina
   NOTE: This step is performed under a dissection microscope.
   1. Incise a hole in the center of the cornea using a 25 G sterile needle.
   2. Cut the cornea, through the hole, in half and enlarge the incision to the sclera and RPE, then remove the sclera and RPE.
   3. Use micro-toothed forceps to gently remove the lens and the vitreous to isolate the neural retina.
   4. Proceed with section 3 to prepare donor retinal suspension or section 4 to prepare donor retinal sheet, as required.

3. Prepare donor retinal suspension (Figure 1)

1. Incubate the neural retina in Papain solution at 37 °C for 20-30 min until no cell clumps are detected.
   NOTE: Gently pipette the cell mixture 15x every 10 min.
2. Follow manufacturer's instructions of the Papain Dissociation Kit to collect single cells.

4. Prepare donor retinal sheets (Figure 1)

NOTE: This step follows section 2 and is performed under a dissection microscope.

1. Put the isolated neural retina cup into a 35 mm Petri dish with prechilled sterile PBS.
2. Use micro scissors to cut the neural retina cup into multiple retinal sheets (around 1 mm x 2 mm).

5. Prepare recipient mice

1. Anesthetize recipient mice with intraperitoneal injection of ketamine (100 mg/kg body weight) and xylazine hydrochloride (20 mg/kg body weight).

2. Confirm the surgical plane of anesthesia by ensuring the loss of blink and pain reflexes, regular breathing, and respiration.
3. Assess the anesthetic depth by the lack of response to the tail pinch or pedal reflexes withdrawal.
4. Always recheck the anesthetic depth during the operative procedure and adjust the anesthetic depth if the animal is responsive to pain. Keep the mice on the pre-warmed surgery table to prevent hypothermia.
5. Dilate recipient pupils with 1% (wt/vol) tropicamide eye drops 5 min before the surgery. A well-dilated pupil can facilitate transpupillary visualization under the operating microscope.
6. Disinfect the operating mouse eye and surrounding ocular tissues with povidone-iodine, wash with sterilized PBS.
7. Apply 0.5% Proparacaine hydrochloride on the mouse eye for analgesia.

6. Subretinal transplantation of retinal grafts (Figure 2)

NOTE: All the following steps were performed under aseptic conditions. Surgical tools were autoclaved (use instrument trays to protect the tool tips and take the plunger out of the micro syringes to prevent them getting stuck in the lumen). Supplier information of the research tools and products is provided in the **Table of Materials**.

1. Anterior chamber paracentesis: Penetrate peripheral cornea into anterior chamber with an insulin needle to allow aqueous humor to passively egress, to prevent the intraocular pressure (IOP) spikes during transplantation.
   NOTE: A successful paracentesis is indicated by the efflux of aqueous humor through the cornea hole.
2. Put a drop of sodium hyaluronate and a glass coverslip (5 mm diameter) on top of the cornea to enable transpupillary visualization of the fundus under the surgical scope.
3. Use toothed forceps to expose the injection locus (1 mm post-corneal limbus) by pressing either the superior or inferior eye wall, as required by the experiment, toward the center of transpupillary vision.
4. Use a microinjection needle to perpendicularly penetrate the sclera.
5. Carefully rotate the needle direction tangentially to allow complete penetration of the sclera-choroid-RPE complex into the subretinal space.
6. Insert the needle tangentially to access the terminal injection locus.
   NOTE: Verify the complete positioning of the needle's bevel by transpupillary vision guidance. Use the superficial retinal vessels as an anatomical reference for accurate needle positioning within the subretinal space.
7. Inject retinal grafts
   NOTE: The presence of small bubbles preloaded in the syringe can facilitate validation of the accurate subretinal positioning of donor cells. Elevated IOP resulting from the subretinal injection increases the risk of donor cell reflux.
   1. If the cornea becomes cloudy[24,25], an indicator of high IOP, keep the needle in the subretinal space for at least 3 min until the cornea clears, an indicator that the IOP has reduced sufficiently.
      NOTE: In rare cases, the IOP may take longer to normalize, and it is recommended keeping the needle in place until the clarity of the cornea is restored even if this would take 8-10 min. Observe

under a surgical microscope to prevent damage to retinal structures.

8. Grasp the injection hole edge with micro-toothed forceps and quickly withdraw the needle.
   NOTE: The criteria for successful subretinal delivery of a retinal cell suspension and sheet are a constant bleb and a visible white sheet in the subretinal space, respectively.
9. Clean the surgical area of the eye with artificial tears and apply ointment if necessary.
10. Post-surgery administration
    1. Place transplanted mice in a pre-warmed (37 °C) clean recovery cage and carefully monitor for distress.
    2. Apply a drop of topical 0.5% Proparacaine Hydrochloride on the surgical eye 2-3 times if distress occurs.
    3. Return mice to the house cage after the mice are completely alert and mobile. Place a small number of food pellets and a gel cup in the cage if the mice have trouble reaching the food hopper.
       NOTE: Keep the eye surface humid until anesthesia wears off to prevent cataract formation.

7. Multimodal confocal scanning laser ophthalmoscopy (cSLO) imaging

1. Two months post-transplantation, anesthetize recipient *Rd1/NS* mice (n=5) by intraperitoneal injection of ketamine (100 mg/kg body weight) and xylazine hydrochloride (20 mg/kg body weight) for imaging.
2. Dilate pupils with 1% (wt/vol) tropicamide eye drops.
3. Perform standard 55° multimodal imaging using the confocal scanning laser ophthalmoscope cSLO system[22].
4. Obtain MR and SD-OCT images using 30 frames of ART averaging.

8. Histological staining

1. Prepare frozen slides of transplanted *Rd1/NS* mice eyes as previously reported [14].
2. Block and penetrate the frozen slides of transplanted retinae using a mixture of 5% goat serum and 1% Triton X100 in PBS.
3. Incubate samples with primary antibodies over night at 4 °C and rinse in PBS (5 min, 3x). The primary antibodies include goat anti-GFP-FITC (1:200), rabbit anti-recoverin (1:1000), and rabbit anti-S-opsin (1:500).
4. Incubate the samples with secondary antibody (goat anti-rabbit Cy3, 1:500) and counterstained with DAPI.
   NOTE: The suppliers of primary and secondary antibodies are listed in **Table of Materials**.

Representative Results

Retinal grafts are successfully delivered into the subretinal space and survived *in vivo*.

The performance of our subretinal transplantation platform was assessed in *Rd1/NS* recipient mice aged 6-8 weeks when their residual retinae were severely thinned due to the almost complete outer nuclear layer (ONL) degeneration. Given the fragility of the retina, the scarcity of cone photoreceptors, and the relatively poor viability of cells in suspension compared to sheet donors, cone-rich mice (*OPN1LW-EGFP;NRL*$^{-/-}$)[14] were used as the sources of donor retinal sheets and rod-rich mice (*Rho::EGFP)* as the

donor for cell suspensions. Cone-rich retinal sheet and rod-rich cell suspensions were successfully delivered into the subretinal space of all recipient mice using our surgical platform, as confirmed by the subretinal bleb and white sheet seen using transpupillary visualization immediately following injection. Two months post-transplantation, multimodal cSLO imaging was performed to track the states of retinal grafts *in vivo*. Cross-sectional OCT scanning showed that retinal grafts survived in the subretinal space and reconstituted the ONL in all recipient mice (**Figure 3A**). Infrared imaging detected no obvious cataracts in all transplanted eyes (**Figure 3B**). Other surgical complications including hemorrhage were rarely detected in transplanted retinae (n = 1/10 eyes) by multicolor reflectance imaging at two months post-transplantation (**Figure 3C**). We performed histological staining to further assess the survival and extent of maturation of photoreceptors in retinal grafts. Abundant cone photoreceptors expressing OPN1LW:EGFP and S-opsin in transplanted retinal sheets were observed. Likewise, transplanted retinal cell suspensions showed a large proportion of Rec^{+} photoreceptors *in vivo*, including numerous $Rho::EGFP^{+}$ rods (**Figure 3D**). The non-transplanted control mice showed severe ONL degeneration with sparse residual cone photoreceptors (Rec^{+}). No EGFP signal was detected in non-transplanted *Rd1/NS* retina (**Figure 3D**).

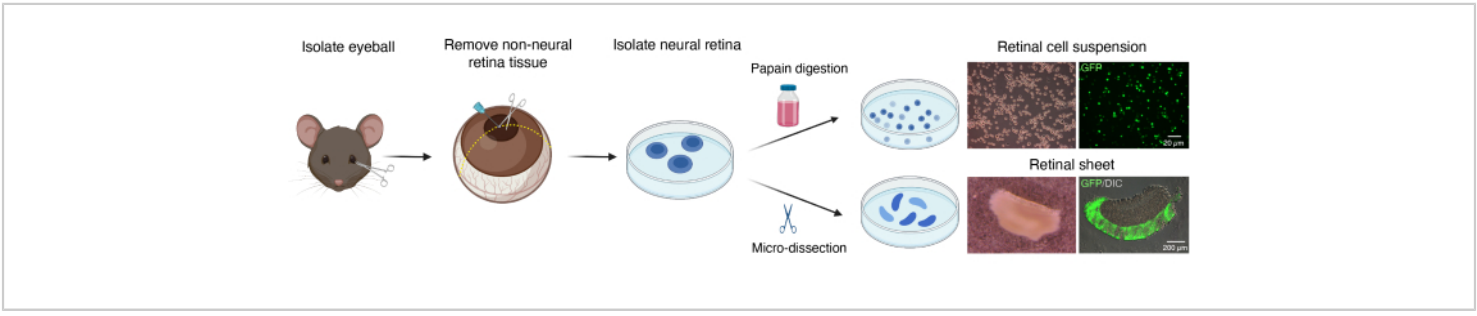

Figure 1: Schematic of collecting donor retinal sheet and cell suspensions. Schematics showing the key steps of collecting retinal cell suspensions and sheets from donor *Rho::EGFP* mice. Bright-field and fluorescent imaging showed representative images of dissociated cells and a dissected sheet isolated from *Rho::EGFP* mice. *Yellow dotted line: incisional margin.* Please click here to view a larger version of this figure.

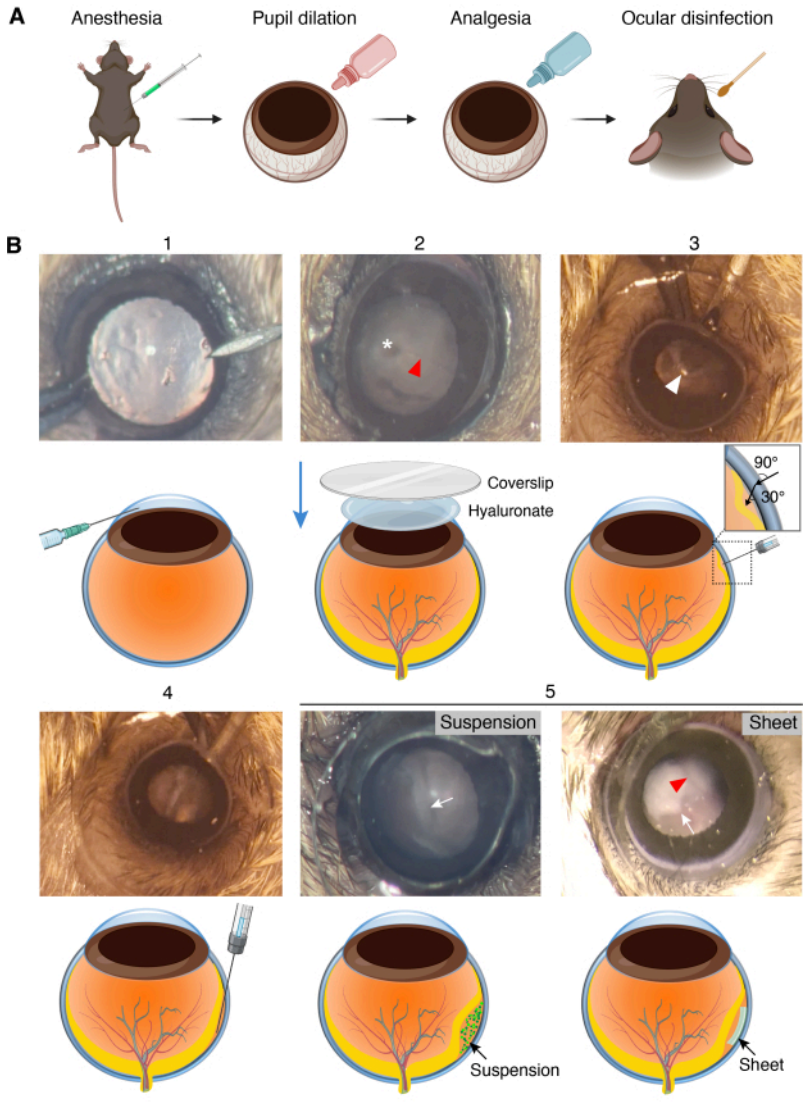

Figure 2: Procedures of subretinal transplantation of retinal sheets and cell suspension in *Rd1/NS* mice.

(**A**) Schematic images of recipient mice preparation. (**B**) Key surgical procedures and corresponding diagrams showing subretinal transplantation of retinal cell suspensions and sheets. Surgical steps include: 1. penetrate the anterior chamber; 2. drop the sodium hyaluronate on the cornea, then mount the coverslip on top of the sodium hyaluronate to facilitate the transpupillary visualization; 3. penetrate outer layers of the eye wall (sclera-choroid-RPE complex). Penetration angles of the needle are on the top right panel of the illustration; 4. insert injection needle; 5. inject grafts. Representative images show the successful delivery of retinal cell suspensions and sheet in two individual recipients, respectively. *Asterisk: optic nerve head; Red arrowheads: retinal blood vessel; White arrowhead: penetrated needle tip; White arrows: grafted retinal suspensions or sheet.* Please click here to view a larger version of this figure.

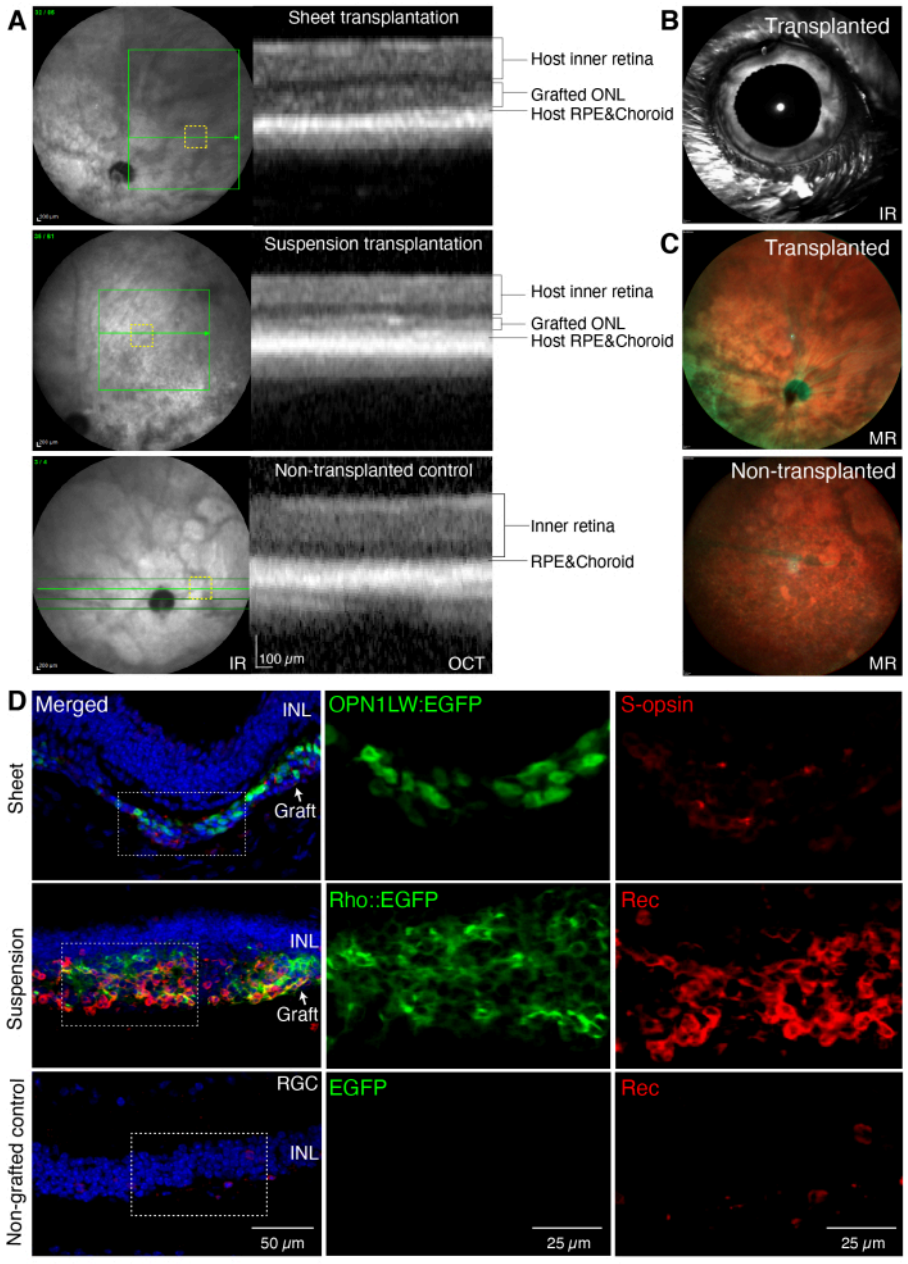

Figure 3: Successful delivery of retinal grafts into the subretinal space and survival *in vivo*. (**A**) Representative SD-OCT images showed the subretinal distribution of transplanted retinal sheets and cell suspensions in two individual *Rd1/NS* mice, respectively. Non-transplanted *Rd1/NS* mice were collected as a control. Region of Interest (ROI) is indicated by yellow-dotted boxes on infrared (IR) fundus images. Inner retina is designated as laminae of retinal ganglion cell layer (RGC), inner plexiform layer (IPL), and inner nuclear layer (INL). (**B**) Representative infrared (IR) image of a transplanted *Rd1/NS* mouse showed no cataract through the dilated pupil. (**C**) Representative multicolor reflectance (MR) images of transplanted and non-transplanted *Rd1/NS* eyes. The transplanted eyes presented no obvious surgical

complications including hemorrhage. (**D**) Immunohistochemistry (IHC) staining of transplanted- (sheet and suspension) and non-transplanted (control) *Rd1/NS* mice. The data showed numerous grafted photoreceptors expressing specific markers of photoreceptors (Rec), rods (Rho::EGFP), L/M cones (OPN1LW:EGFP), and S-cones (S-opsin) at two months post-transplantation. Recipient retinal laminae were identified by DAPI staining (blue). Retinal grafts were identified by EGFP reporter (green). The non-transplanted mice retina showed severe ONL degeneration with sparse residual cone photoreceptors (Rec^{+}). No EGFP signal was detected in non-transplanted *Rd1/NS* retinae. Magnified images were presented on the right two panels. *Abbreviations: RGC: retinal ganglion cell layer; INL: inner nuclear layer. ONL: outer nuclear layer.* Please click here to view a larger version of this figure.

Discussion

Subretinal transplantation in mice is technically challenging due to the small size of mouse eyes. In this study, we developed a simple and reproducible platform for subretinal transplantation in mouse recipients. The platform allows consistency in donor viability protection, successful subretinal delivery, and ensures a low complication rate.

The subretinal transplantation technique depicted here was developed based on the trans-scleral route, where the injection needle penetrates the outer layers (sclera-choroid-RPE complex) of the eyewall. Compared to the trans-corneal[19,20] and trans-vitreous[21,26,27] approach, the trans-scleral approach directly enters the subretinal space without penetrating the anterior segment structures and neural retina, thus enabling a harmless transplantation and reducing the risk of donor cell reflux through the penetrating hole. A challenge of the trans-scleral approach is to ensure the needle tip propagates along the subretinal space before arriving at the terminal delivery site, while avoiding potential disturbance of the adjacent RPE and choroid layers. It is challenging to achieve these goals through a traditional trans-scleral approach[28,29] without direct visual guidance. In this study, we developed a transpupillary vision-guided platform using a surgical microscope and external illumination of the retina to facilitate subretinal transplantation in mouse models. The platform allows the operator to track the surgical process and the recipients' retinal condition by providing real-time visualization of the recipient fundus under the surgical microscope. For example, successful penetration of the sclera-choroid-RPE complex can be simply validated by a relatively bright reflectance of the needle tip via transpupillary visualization. Importantly, when guided by transpupillary visualization, the operator can accurately modulate the angle and the depth of injection according to the relative position between the needle and anatomic structures in the recipient retina (e.g., retinal vessels and optic nerve head). Moreover, accurate administration of materials using this technique can minimize RPE, choroid trauma, and other surgical complications. Indeed, we found hemorrhages can be minimized by avoiding maneuvers in close proximity to retinal blood vessels with the help of transpupillary visualization.

Reflux of donor cells is a major cause of surgical failure post-injection[29,30]. There are multiple factors involved in promoting donor cell reflux. The high intraocular pressure (IOP) of the recipient eyes caused by the injected donor cell mixtures is an important factor that promotes the reflux of cells out of the eye. Our protocol reduces the recipients' IOP by anterior chamber penetration at the beginning of the surgery and enables the IOP to lower spontaneously by aqueous fluid

egress before pulling out the vitreous needle from the vitreous cavity. A second factor is the unsealed injection tunnel in the recipients' eyes. To prevent graft reflux back through the injection tunnel, we use a small size micro-injection needle (34G) to create a self-sealing tunnel when penetrating the eyewall and toothed micro-forceps to hold the edges of the external tunnel opening when pulling out the needle. In conventional transplantation, refluxed grafts are hardly detected because they may occur rapidly or dissipate quickly. The sodium hyaluronate, upon application of the coverslip, almost invariably slides off the cornea to cover most of the limbus and sclera including the sclerotomy site. Following cell injection, the sodium hyaluronate at the sclerotomy site can help to slow down the bulk flow of refluxed contents, if any, and help to make them visually detectable under the surgical microscope. Moreover, the absence of reflux can also be verified by tracing the constancy of the subretinal bleb for cell suspensions and the intraocular location of the retinal sheet using our protocol, which could be useful for laboratories where optical coherence tomography (OCT) is unavailable.

While a substantial number of photoreceptor cells survived in the transplanted retinal sheets, no discernible cellular orientation, or sheet orientation, could be detected. Although delivery of 3D retinal sheets to large animals has been established[26,31,32], the subretinal space in mice, coupled with the lack of intraoperative retinal imaging capability, makes it very challenging to ensure the maintenance of sheet orientation intraoperatively as well as in the immediate postoperative period during which the transplanted cells or sheets may become unstable as the mouse regains ambulation.

We describe a trans-scleral surgical platform with direct transpupillary vision guidance for the subretinal transplantation in mouse recipients. This platform enables high accuracy of injection and a low rate of surgical complications. This platform enables precise delivery of known doses of cells, is relatively easy to learn, and facilitates subretinal delivery in addition to intra-retinal or intravitreal injections for different types of therapeutic agents including gene therapy.

Disclosures

MSS is/was a paid advisor to Revision Therapeutics, Johnson & Johnson, Third Rock Ventures, Bayer Healthcare, Novartis Pharmaceuticals, W. L. Gore & Associates, Deerfield, Trinity Partners, Kala Pharmaceuticals, and Acucela. MSS receives sponsored research support from Bayer. These arrangements have been reviewed and approved by the Johns Hopkins University in accordance with its conflict-of-interest policies. KVL is named as inventors on patent applications at University of Colorado. MSS and YVL are named as inventors on patent applications at Johns Hopkins University.

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References

1. Mehat, M. S. et al. Transplantation of human embryonic stem cell-derived retinal pigment epithelial cells in macular degeneration. *Ophthalmology.* **125** (11), 1765-1775 (2018).
2. da Cruz, L. et al. Phase 1 clinical study of an embryonic stem cell-derived retinal pigment epithelium patch in age-related macular degeneration. *Nat Biotechnol.* **36** (4), 328-337 (2018).
3. Kashani, A. H. et al. A bioengineered retinal pigment epithelial monolayer for advanced, dry age-related macular degeneration. *Sci Transl Med.* **10** (435) (2018).
4. Schwartz, S. D. et al. Embryonic stem cell trials for macular degeneration: a preliminary report. *Lancet.* **379** (9817), 713-720 (2012).
5. Mandai, M. et al. Autologous induced stem-cell-derived retinal cells for macular degeneration. *N Engl J Med.* **376** (11), 1038-1046 (2017).
6. Sharma, R. et al. Clinical-grade stem cell-derived retinal pigment epithelium patch rescues retinal degeneration in rodents and pigs. *Sci Transl Med.* **11** (475) (2019).
7. Singh, M. S. et al. Reversal of end-stage retinal degeneration and restoration of visual function by photoreceptor transplantation. *Proc Natl Acad Sci U S A.* **110** (3), 1101-1106 (2013).
8. Shirai, H. et al. Transplantation of human embryonic stem cell-derived retinal tissue in two primate models of retinal degeneration. *Proc Natl Acad Sci U S A.* **113** (1), E81-90 (2016).
9. Ghosh, F., Wong, F., Johansson, K., Bruun, A., Petters, R. M. Transplantation of full-thickness retina in the rhodopsin transgenic pig. *Retina.* **24** (1), 98-109 (2004).
10. Klassen, H. et al. Progenitor cells from the porcine neural retina express photoreceptor markers after transplantation to the subretinal space of allorecipients. *Stem Cells.* **25** (5), 1222-1230 (2007).
11. Das, T. et al. The transplantation of human fetal neuroretinal cells in advanced retinitis pigmentosa patients: results of a long-term safety study. *Exp Neurol.* **157** (1), 58-68 (1999).
12. Humayun, M. S. et al. Human neural retinal transplantation. *Invest Ophthalmol Vis Sci.* **41** (10), 3100-3106 (2000).
13. Liu, Y. et al. Long-term safety of human retinal progenitor cell transplantation in retinitis pigmentosa patients. *Stem Cell Res Ther.* **8** (1), 209 (2017).
14. Liu, Y. V. et al. Characterization and allogeneic transplantation of a novel transgenic cone-rich donor mouse line. *Exp Eye Res.* **210**, 108715 (2021).
15. Pearson, R. A. et al. Restoration of vision after transplantation of photoreceptors. *Nature.* **485** (7396), 99-103 (2012).
16. Ortin-Martinez, A. et al. Photoreceptor nanotubes mediate the in vivo exchange of intracellular material. *EMBO J.* **40** (22), e107264 (2021).
17. Zou, T. et al. Organoid-derived C-Kit(+)/SSEA4(-) human retinal progenitor cells promote a protective retinal microenvironment during transplantation in rodents. *Nat Commun.* **10** (1), 1205 (2019).
18. Singh, M. S. et al. Transplanted photoreceptor precursors transfer proteins to host photoreceptors by

a mechanism of cytoplasmic fusion. *Nat Commun.* **7**, 13537 (2016).

19. Qi, Y. et al. Trans-corneal subretinal injection in mice and its effect on the function and morphology of the retina. *PLoS One.* **10** (8), e0136523 (2015).
20. Nickerson, J. M. et al. Subretinal delivery and electroporation in pigmented and nonpigmented adult mouse eyes. *Methods Mol Biol.* **884**, 53-69 (2012).
21. Fang, Y. et al. Safety evaluation of subretinal injection of trypan blue in rats. *Eur Rev Med Pharmacol Sci.* **22** (10), 2923-2933 (2018).
22. Liu, Y. V. et al. Quantifiable in vivo imaging biomarkers of retinal regeneration by photoreceptor cell transplantation. *Transl Vis Sci Technol.* **9** (7), 5 (2020).
23. Liu, Y. V. et al. Single-cell transcriptome analysis of xenotransplanted human retinal organoids defines two migratory cell populations of nonretinal origin. *Stem Cell Reports.* **18** (5), 1138-1154 (2023).
24. Park, S. et al. Animal models of corneal endothelial dysfunction to facilitate development of novel therapies. *Ann Transl Med.* **9** (15), 1271 (2021).
25. Li, X. et al. Acute ocular hypertension disrupts barrier integrity and pump function in rat corneal endothelial cells. *Sci Rep.* **7** (1), 6951 (2017).
26. Chao, J. R. et al. Transplantation of human embryonic stem cell-derived retinal cells into the subretinal space of a non-human primate. *Transl Vis Sci Technol.* **6** (3), 4 (2017).
27. Singh, R. K., Occelli, L. M., Binette, F., Petersen-Jones, S. M., Nasonkin, I. O. transplantation of human embryonic stem cell-derived retinal tissue in the subretinal space of the cat eye. *Stem Cells Dev.* **28** (17), 1151-1166 (2019).
28. Zhao, C. et al. Development of a refined protocol for trans-scleral subretinal transplantation of human retinal pigment epithelial cells into rat eyes. *J Vis Exp.* (126),e55220, (2017).
29. Wert, K. J., Skeie, J. M., Davis, R. J., Tsang, S. H., Mahajan, V. B. Subretinal injection of gene therapy vectors and stem cells in the perinatal mouse eye. *J Vis Exp.* (69), e4286, (2012).
30. Wongpichedchai, S., Weiter, J. J., Weber, P., Dorey, C. K. Comparison of external and internal approaches for transplantation of autologous retinal pigment epithelium. *Invest Ophthalmol Vis Sci.* **33** (12), 3341-3352 (1992).
31. Luo, Z. et al. Establishing a surgical procedure for rhesus epiretinal scaffold implantation with HiPSC-derived retinal progenitors. *Stem Cells Int.* **2018**, 9437041 (2018).
32. Luo, Z. et al. Biodegradable scaffolds facilitate epiretinal transplantation of hiPSC-Derived retinal neurons in nonhuman primates. *Acta Biomater.* **134** 289-301 (2021).