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Transplantation of Photoreceptors for Restoration of Sight

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14. ABSTRACT

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Upon successful surgery, we observed no rejection of the retina/RPE transplant under the degenerated retinas. Photoreceptors survived only when RPE was included in the transplanted sheet. Ganglion cell layer in the transplant completely disappeared over a few weeks post-op, and the inner plexiform layer thinned-down to about 30% of its initial thickness. However, inner nuclear layer and photoreceptors remained preserved, including up to 6 layers of photoreceptor nuclei with their inner and outer segments. Immunostaining of the rod bipolar cells revealed some evidence of the dendrites sprouting from the host bipolar cells and synaptogenesis with bipolar cells in the transplant.

Despite the long-term survival of the transplanted photoreceptors with RPE in rats with retinal degeneration and some morphological indications of reconnection to the host retina, electrophysiological recordings (visually evoked potentials) did not demonstrate restoration of visual sensitivity in the retina.

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1. Abstract

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2. Accomplishments

Introduction

Loss of photoreceptors in inherited retinal diseases or age related macular degeneration (AMD) can lead to blindness. Transplantation of photoreceptors is one of the most natural ideas when thinking about restoration of sight in such conditions. First, human retinas can be harvested several hours post mortem and kept healthy in culture for days [1]. Second, the laminar organization of the retina allows relatively simple implantation of the sheet graft in the subretinal space. Third, the immune privilege of the subretinal space [2-4] helps reducing the chance of rejection.

Efforts towards retinal transplantation started with the assumption that only fetal retinas can survive and be plastic enough to reconnect to the host retina. Survival of the fetal rat retina in the anterior chamber of the maternal eye was demonstrated as early as 1959 [5]. More recent experiments demonstrated survival of the fetal retinal grafts in the subretinal space and some evidence of reconnection between the host and the transplant [6-9].

Based on those encouraging results, retinal sheets from unborn fetuses were transplanted into several patients with retinitis pigmentosa (RP) and AMD [10-14]. Majority of Radtke's patients showed an improvement of visual acuity in the transplanted eye, with the best improvement from 20/800 to 20/200 over 5 years [14]. Despite this proof of concept, ethical concerns with the use of unborn fetuses [15] and limited availability of the fetal tissue precluded adaptation of this approach. Another limitation of the fetal graft is that the ganglion cell layer does not disappear after transplantation and prevents effective connectivity between the donor and the host retinas [7].

Since the earliest description of fetal retinal plasticity in transplantation [16], several approaches involving photoreceptor precursors from stem cells were developed. Some signs of survival and integration [17] were initially demonstrated, but in a limited number of cells [18, 19]. However, integration and formation of outer segments was observed only when at least a fraction of the host outer nuclear layer (ONL) was still present, but did not exhibit normal and uniform polarization when injected in dystrophic retinas [20]. Results from that study were later attributed to host photoreceptor cells contaminated by intercellular transfer of cytoplasmic content from the fluorescently labelled cells, rather than integration [21].

While the synaptic plasticity in the donor photoreceptors is essential for the success of the graft, the host bipolar cells must also be able to form new synaptic contacts. Interestingly, following local photoreceptor coagulation by laser, adult bipolar cells that have been deprived from presynaptic inputs have been shown to reach out tens of microns away from the lesion to establish new functional connections with photoreceptors [22, 23].

Full thickness retinal transplantation has been also performed using neonatal donor retina and stem cells-derived 3D retinal organoid grafts [24, 25]. These grafts are not fully differentiated at the time implantation. 3D retinal organoids exhibited signs of dysfunctional and non-directional photoreceptors, with the formation of rosettes [25]. Transplantation of fetal photoreceptors coupled with RPE has provided encouraging results in animals [26] and humans [13] with degenerate retina. While the presence of RPE on those transplants was beneficial, ethical concerns and limited availability of the fetal tissue still precluded adaptation of this approach.

Transplantation of mature retinal sheets without RPE in a light-induced degeneration model demonstrated better structural integrity of the outer retina when transplanted with the inner retina, compared to transplantation of the photoreceptors alone [27]. However, the absence of RPE in these grafts reduces the translational applicability of the technique, as it would not be suitable for patients having dysfunctional RPE in diseases such as AMD.

We investigated here the feasibility of mature retinal allografts transplanted together with RPE in two different models of retinal degeneration. In the first model, the Royal College of Surgeon (RCS) rat, a mutation in the MERTK gene reduces the phagocytic capability of the RPE, leading to degeneration of photoreceptors by 4 months [28]. While it's one of the forms of Retinitis Pigmentosa, this condition would require transplantation of not only photoreceptors but also functional RPE, thereby mimicking requirements for treatment of geographic atrophy. The S334ter-3 rats were used as an alternative model, where photoreceptors degenerate by 2 months due to a mutation in the rhodopsin, while the RPE remains fully functional [29].

Methods

Experimental Design

This study was designed to evaluate the outcomes of mature photoreceptors transplantation from healthy rats into 2 different rat models of retinal degeneration. Following the development of a surgical technique, we included in this analysis all the transplants which

displayed a discernable layering on OCT, 1 week after transplantation. Therefore, this study does not describe the success rate of the transplantation procedure itself, but rather the extent of tissue integration, when the graft is not rejected.

Animals

Rats with retinal degeneration were obtained from the colonies of Royal College of Surgeons (RCS, P139-P257, n=6) or S334ter-3 (source RRRC, strain SD-Tg(S334ter)3Lav, P72 – P90, n=7) maintained at the Stanford Animal Facility. Long-Evans (LE, P18 – P50, n=8) rats were purchased from Charles River (Wilmington, MA). All animals were housed in a 12-hour light/12-hour dark cycle, with food and water ad libitum. All experimental procedures were conducted in accordance with the institutional guidelines and conformed to the Statement for the Use of Animals in Ophthalmic and Vision research of the Association for Research in Vision and Ophthalmology (ARVO).

Graft preparation

Properly designed and performed transplantation procedure is one of the key factors in success of the graft (Fig. 1 and Video 1). Donor rats (Long Evans, n=8) were deeply anesthetized with a mixture of ketamine (75mg/kg) and xylazine (5mg/kg) and euthanized with an intracardiac injection of Beutanesia (0.5mL). Eyes were enucleated and placed in oxygenated Ames's solution (SIGMA) at room temperature for further dissection. The anterior segment was removed and the lens was slowly pulled away, along with the vitreous. If some vitreous was still present on the retinal side, it was removed using forceps. One-millimeter diameter biopsy punch was used to isolate the transplant. At that time, most RPE cells stayed attached to the retina (Fig. 2B). The transplant was kept in oxygenated Ames solution until the donor was ready – typically 10-15min. It was then loaded into a custom-made hydraulic tool (Fig.2C), having a 200µm-high, 1mm-wide micro-channel connected to a syringe with viscoelastic gel (Viscoat, Alcon).

Transplantation

As a recipient, we used two animal models of retinal degeneration: the Royal College of Surgeon (RCS) rat and the S334ter-3 rat. Both models exhibit complete photoreceptor degeneration with the difference of dysfunctional RPE in RCS rats and fully functional RPE in S334ter-3 rats.

Recipient rats were anesthetized with a mixture of ketamine (75mg/kg) and xylazine (5mg/kg), topical anesthetics (Tetracaine, 0.5%). Dilation drops (Phenylephrin 2.5%, tropicamide 1%) were applied to the operated eye. The recipient eye was prepared with a transscleral incision followed by retinal detachment with BSS and viscoelastic injection in the subretinal space (Fig. 2D), followed by insertion of the transplant into the subretinal space under visual control (Fig. 2E). Balanced salt solution (BSS) was then injected into the vitreous cavity to reattach the host retina over the transplant. Placement of the graft and reattachment of the host retina was verified with Optical Coherence Tomography (OCT) (Suppl. Fig. 1F-G).

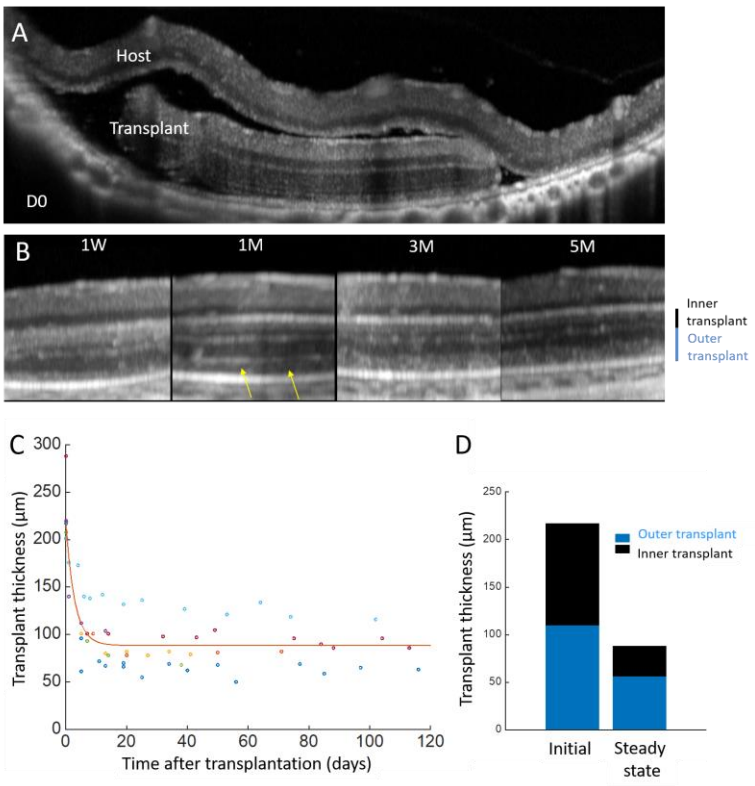

Figure 1. In-vivo evaluation of the transplant's structural preservation. A) OCT of the transplanted retina over time. One week (1W) after transplantation (D0), the host retina has reattached and maintains its laminar structure. After 1 month (1M), the outer segments of the transplant have grown back (yellow arrows) while its inner retina has thinned and this structure is still maintained at 3 months. B) Time course of the transplant thickness, up to 4 months, averaged over 8 animals (dots colored per animal). The data was fitted with an exponential function (red line), $y=y_\infty+(y_0-y_\infty)\cdot e^{-t/\tau}$ with y_∞=88±8 µm, y_0=217±18 µm and τ=3±1 days (± indicate the 95% confidence interval). Note that animals were terminated at different time points, but all of them reached the steady state (t>4τ). C) Initial and steady-state thickness of the inner and outer layers of the transplant. The inner layers (GCL, IPL, INL) of the transplant decreased from 107µm to 32µm, while the outer layers (OPL, ONL, OS) decreased less - from 110µm to 56µm.

OCT imaging

Spectral-domain OCT images were obtained by using HRA2-Spectralis (Heidelberg Engineering), with the cornea covered with viscoelastic and a coverslip to cancel its optical power (Fig. 2F-G). Pupils were fully dilated and throughout the procedure, 1% methylcellulose was used to maintain corneal clarity. Transplanted rats were

followed from day 0 until sacrifice. ONL layer thickness was measured at the center of the transplant.

Histology

For histology, animals were sacrificed at D1, D13 and D42. Prior to enucleation, the superior edge of the eye was marked under deep anesthesia. Both eyes of each animal were enucleated and fixed in 1% paraformaldehyde and 1.25% glutaraldehyde fixative prepared with 5mM calcium chloride and 5% sucrose for 24 hours at room temperature. The cornea and lens were removed, leaving a posterior eye cup, which was dehydrated through a graded series of alcohols, infiltrated in propylene oxide, and embedded in epoxy. The 0.5µm-thick sections were taken using Reichert Ultracut E, stained with 0.5% toluidine blue, and serial sections of the slides were examined by light microscopy.

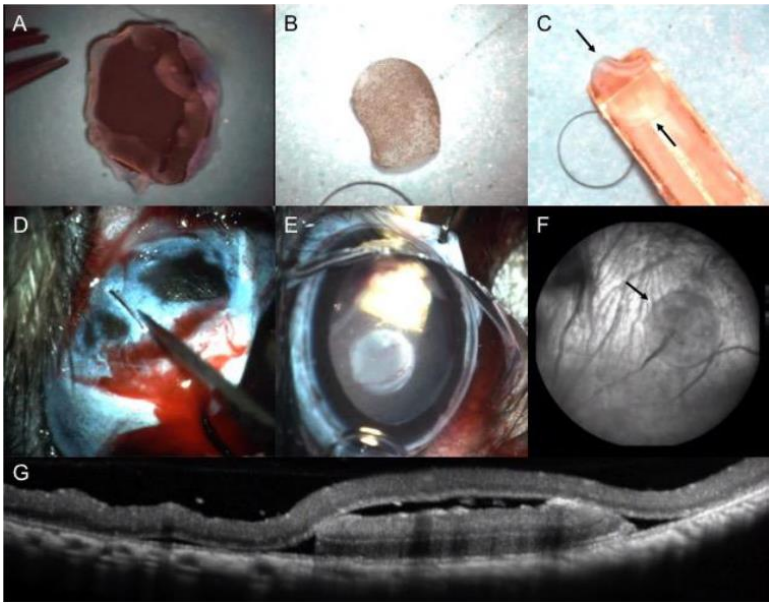

Figure 2. Tissue preparation and implantation.

(A) The eyecup from the WT donor is isolated after removal of the anterior segment, crystalline lens, and the vitreous. B) A 1mm piece of retina is isolated using a biopsy punch. Pressure during the procedure usually allows keeping majority of the RPE cells attached to photoreceptors. C) Tissue is embedded in viscoelastic gel and loaded into a custom-made implantation tool (black arrows show the boundaries of the transplant). In the recipient, sclerotomy and retinotomy are performed to relieve some of the intraocular pressure (D). The transplant is inserted into the subretinal space under visual control (E). After releasing the transplant, some BSS is injected into the vitreous cavity to facilitate retinal reattachment and stabilize the transplant. Surgical outcomes are assessed using infrared fundus imaging (F) and optical coherence tomography (G). See Video 1 for more details.

Immunohistochemistry

For immunohistochemistry, animals were sacrificed at 1, 2, 4 and 6 months. Before enucleation, the superior edge of the eye was marked under deep anesthesia. Both eyes of each animal were enucleated and fixed in 4% paraformaldehyde overnight. The cornea and lens were removed, and the eyecup was embedded in the Optimal Cutting Compound, frozen and sectioned with 12µm thickness. The sections were permeabilized with triton incubated overnight with the following antibodies – (PKC, Santa Cruz Biotechnologies, SC-8393, 1:100 - Bassoon, Stressgen,

VAM-PS003, 1:1000 - Cone, Arrestin Millipore, AB15282, 1:1000) and 2 hours in secondary antibodies (Alexa488 – Alexa594, R37114, A11058, A21206, 1:100) with DAPI. Sections were imaged using confocal microscope (Zeiss, LSM780) and Airyscan (Zeiss, LSM880).

Statistical analysis

The layers thickness in the transplants was measured *in-vivo* using OCT at various time points in 8 animals, and then histologically at the end of the follow-up period. The exponential fit was obtained on all the data points, and the 95 % confidence interval was calculated using the Matlab fitting toolbox (The Mathworks). Length of the glial seal (the hyper-reflective layer between the host and transplant) was measured on the central OCT section at the last time point of the follow-up for each animal.

Electrophysiology (visually evoked potentials)

For VEP recordings, three skull screw electrodes are implanted, as published previously[30], and secured in place with cyanoacrylate glue and dental acrylic. Two electrodes are placed over the visual cortex, one on each hemisphere, 4 mm lateral from midline, 6 mm caudal to the bregma. A reference electrode is implanted 2 mm right to the midline and 2 mm anterior to the bregma. Nose and tail needle electrodes served as another reference and the ground, respectively. Recordings start 2 weeks after the implantation. In these measurements, animals are anesthetized after 12-hour overnight dark adaptation. Pupils are fully dilated with 1% tropicamide and 2.5% phenylephrine solutions, and cornea is lubricated with 1% methylcellulose.

Using the Dome stimulation with Espion E2 electrophysiology recording system (Diagnosys LLC), flashes of 4ms are delivered to both eyes simultaneously within 10-second inter-stimulus intervals, and acquired waveforms are averaged. For multifocal recordings, a binary sequence of black and white squares (20x20) is generated using Matlab (The Mathworks, Psychtoolbox) and displayed in front of the animal from a 21-inch liquid crystal display (LCD), with 60Hz refresh rate. One square element of 2.87cm in width corresponds to 13.7 deg of the visual angle. Each frame is presented for 100ms, followed by a black screen for 400ms (2 Hz image refresh rate). The mfVEP signals are then analyzed offline with a custom Matlab script.

Results

Since retinal ischemia and severing the ganglion cell axons cause the loss of the inner retinal neurons [31-33], while photoreceptors in these conditions survive due to choroidal circulation, we decided to transplant mature full-thickness retinas, with the hypothesis that only the outer retina would survive after the transplantation. We developed and implemented a surgical procedure that minimizes damage to both host and donor tissue (Fig. 2 and Video 1). Survival of the transplant over time was assessed with optical coherence tomography (OCT, Fig. 1A-B), by monitoring the thickness of the retinal layers. Transplants without RPE, as well the ones that underwent very traumatic surgery, showed a rapid (<1 week) loss of photoreceptors. However, when RPE cells stayed attached to the retinal transplant, we observed long-term survival of the photoreceptors in both models of retinal degeneration. Thickness of the transplant rapidly (τ=3±1days) decreased from 217±18µm to 88±8µm (± values indicate 95% confidence interval, n=8), and remained stable after that (Fig. 1C). As expected, most of the thinning was in the inner layers (ganglion cell layer, GCL; inner plexiform layer, IPL; and inner nuclear layer, INL) of the transplant: from 109±6µm to 31±4µm (-70%, Fig. 1D), while the outer layers (outer plexiform layer, OPL; outer nuclear layer, ONL; and inner/outer segments, IS/OS) thinned by about 50%.

Histology confirmed the atrophy of the inner retinal layers of the transplant as early

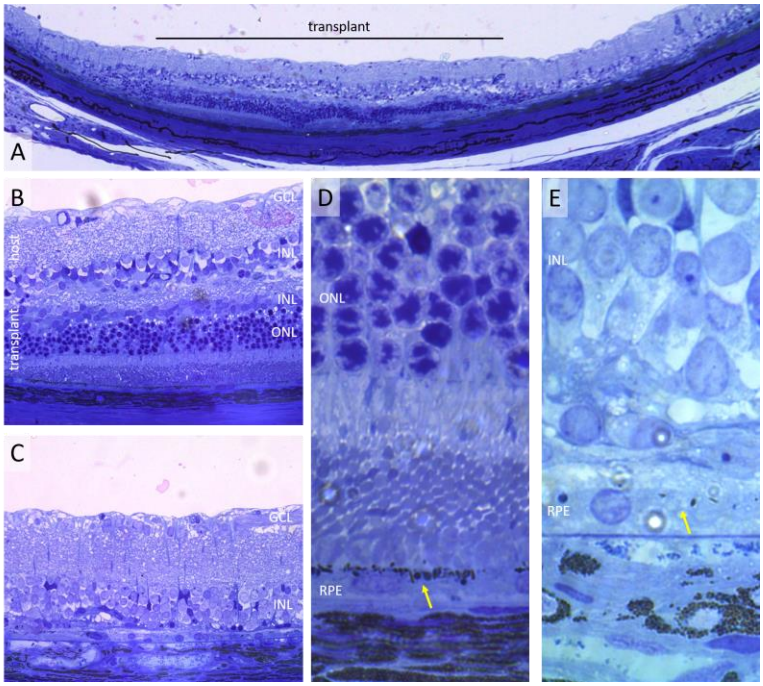

Figure 3. Histology of transplanted retina 2 weeks post-op.
A) Wide view of the transplanted retina (Long Evans transplanted into RCS rat) showing the 1mm transplant (black line) and the control area outside it. B-C) Higher magnification of the same sample showing excellent preservation of the transplanted photoreceptors (B), compared to the area outside the transplant (C). The inner retina of the transplant is significantly thinned, with no distinct ganglion cell layer. D-E) The close-up illustrates pigmented RPE monolayer under the transplant (yellow arrow - D), as opposed to non-pigmented RPE in a control area (E).

as 2 weeks after transplantation (Fig. 3). At the same time, photoreceptors remained well-preserved, with 6-7 layers of the nuclei in the ONL, and well-defined inner/outer segments. Disappearance of the RGC layer of the transplant, likely due to severance of the axons, brought the IPL of the transplant in direct contact with the INL of the host.

In RCS rats, RPE cells cannot properly recycle the photoreceptor outer segments, leading to accumulation of subretinal debris and the subsequent loss of photoreceptors [28]. Following co-transplantation of RPE along with the retina, we observed the presence of pigmented endosomes in the RPE layer (Fig. 3D), indicating some phagocytic function [34], as opposed to lack of pigment in the non-transplanted area. The transplanted RPE appeared as a monolayer rather than an additional layer on top of native RPE, either due to fusion of the

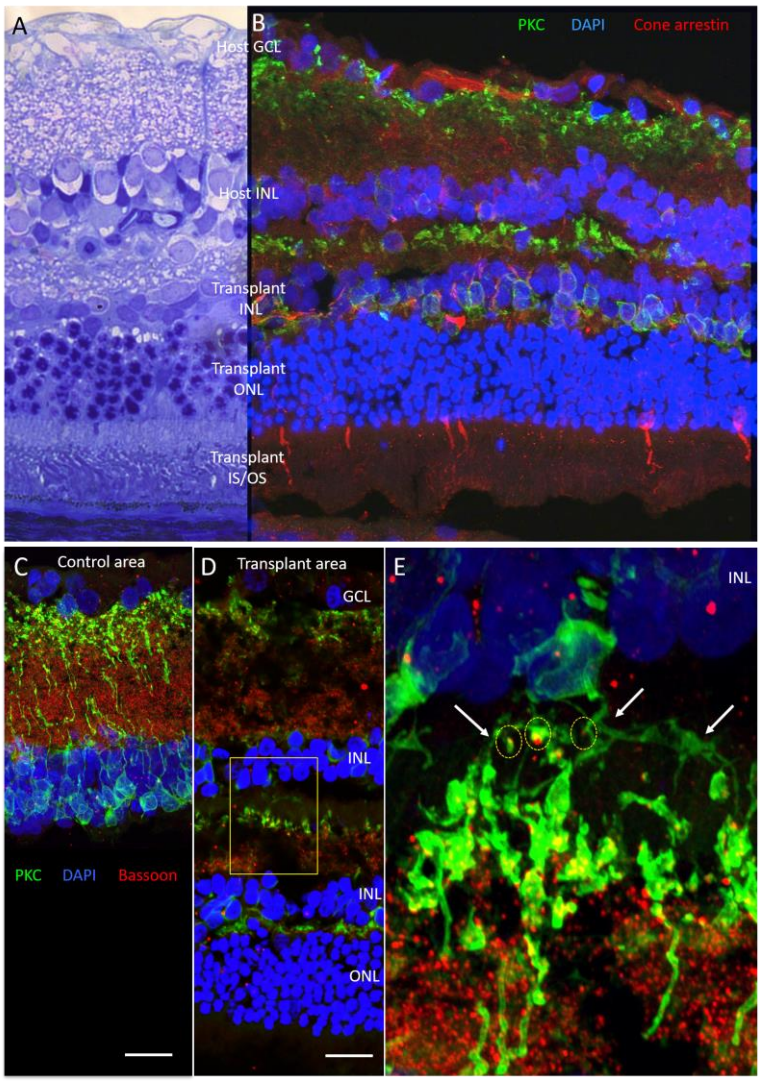

Figure 4. Photoreceptors preservation and synaptic connections between the host and transplanted bipolar cells.

A) Histology shows excellent preservation of the photoreceptors, including their inner and outer segments 13 days after surgery (RCS rat transplanted from a Long Evans donor). In this example, there is no insulating layer between the transplant and the host, and tissues seem to have fused. B) Confocal image of another sample (S334ter-3 transplanted from a Long Evans) 61 days after surgery. Cone arrestin (red) shows cone photoreceptors with their outer segments. PKCα (green) and DAPI (blue) show the plexiform and nuclear layers in the transplant and the host retina. C) Non-transplanted and (D) transplanted areas stained with PKC (rod bipolar cells), Bassoon (presynaptic ribbon synapses) and DAPI. E) Close-up of the area between the host and the transplant, where bipolar cells from the host (top INL) send dendrites into the inner plexiform layer of the transplant (white arrows), which co-localize with presynaptic bassoon staining (red), as outlined with the yellow circles.

two layers or disruption of the initial RPE during subretinal surgery. This second hypothesis would explain why even in S334ter rats, where RPE is functional, we found that retinal transplants without RPE did not survive.

In majority of samples, the periphery of the transplant displayed a high degree of integration, with 53.7±10 % (s.e.m.) of its length having no visible boundary between the host and transplant (Fig. 4A), and excellent preservation of the photoreceptor inner and outer segments. Immunohistochemistry demonstrated layered structure in both the transplant and the host retina (PKCα in rod bipolar cells, Fig. 4C-D), as well as preservation of the cone outer segments (cone Arrestin, Fig. 4B).

Confocal microscopy showed that, unlike the control area, where no vertical extension of the bipolar cell dendrites could be observed (Fig. 4C), in the transplanted areas, dendrites were extending from the host bipolar cells into the inner plexiform layer of the transplant (Video 2). Co-localization of the dendritic tips of the host rod bipolar cells with bassoon presynaptic marker (Fig. 4D-E) indicated the synaptogenesis between the bipolar cells of the host and the transplant. Such connection occurred predominantly near the edges of the transplant. To eliminate the possibility of the co-localization being ectopic contacts, future work will be centered on electron microscopy, electrophysiological and behavioral studies after transplantation.

Some samples displayed even higher degree of integration, with a complete fusion between the inner retina

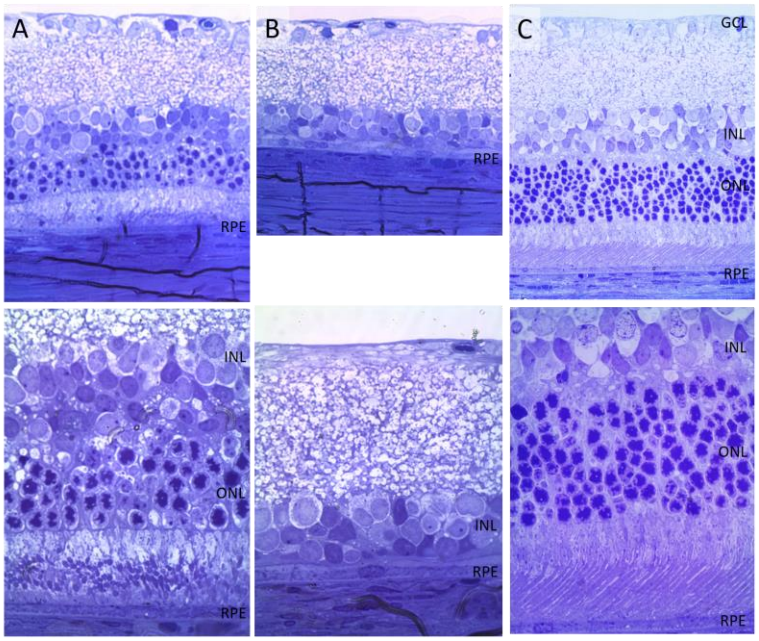

Figure 5. Histology of a transplanted retina at 6 months post-op, compared to control and to wild type retina.
A) The transplanted area has nearly normal anatomy, with a single inner nuclear layer and transplanted photoreceptors merging with the host retina (from a Long Evans into S334ter rat). B) The non-transplanted area completely lacking photoreceptors. C) Healthy Long Evans rat retina is shown here for comparison. Top row is imaged via x40 objective, bottom row – via x100.

of the host and the transplant (Figure 5). Such integration demonstrates that in ideal conditions, the ischemia produced by the transplant harvesting can indeed result in a loss of its inner retina, bringing the grafted photoreceptors into direct contact with the host bipolar cells.

In other samples, a GFAP-positive layer was observed in the center of the transplant, which prevented cell processes from reaching the other side (Figure 6). This layer appeared in OCT as a hyper-reflective membrane developing over time, and could be due to the initial presence of the inner limiting membrane (ILM). Indeed, this membrane has been shown to serve as a scaffold for cellular proliferation in retinal pathologies [35]. This issue could be overcome by ILM peeling from the donor tissue prior to transplantation. It is also possible that direct connection between the host bipolar cells and transplanted photoreceptors could be facilitated by prior removal of the inner retina

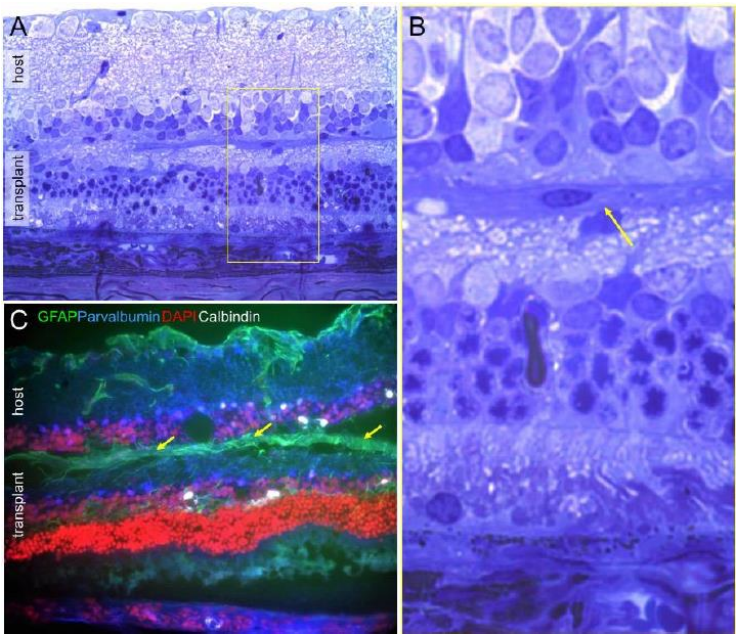

Figure 6. Insulating layer at the center of some transplants. A-B) Histology of transplanted retina (RCS transplanted for 6 weeks from LE donor) showing a seal layer between the transplant and the host at its center. Some fibrotic cells seem to contribute to formation of this layer (yellow arrow). C) Thick insulating layer (yellow arrows) in the center of the transplant (LE into S334ter-3, 61 days post-op) exhibits GFAP (green). Other retinal layers in this frozen section are shown with Parvalbumin (blue), DAPI (red) and Calbindin (white) staining.

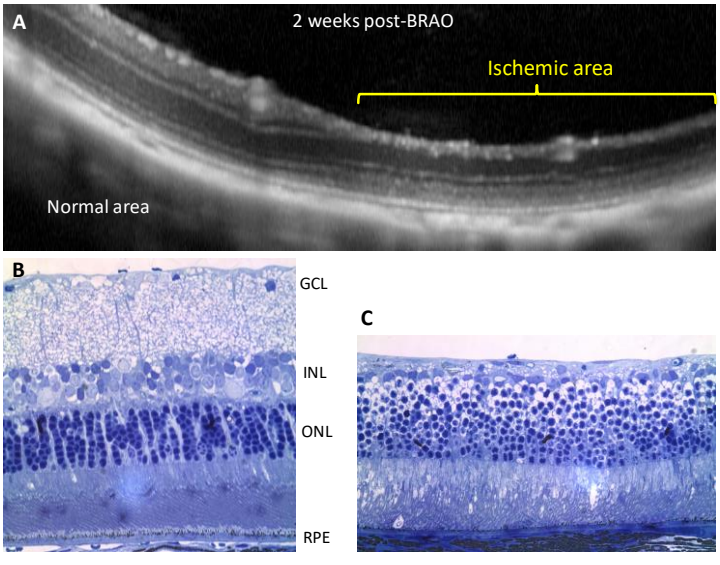

Figure 7: Ischemia of the inner retina 2 weeks post occlusion of the artery. A) OCT showing transition between normal (left) and ischemic areas (right). B) Histology in the normal area. C) Histology in ischemic area.

using retinal artery occlusion a few weeks before harvesting.

We also explored the idea that integration of the transplant with the host might be facilitated by prior removal of the inner retina using retinal artery occlusion a few weeks before harvesting [36], as shown in Fig. 7. In this procedure, Rose Bengal (50mg/kg) is injected peritoneally, and a pulse of 532nm laser is applied to the branch retinal artery under direct observation via a slit lamp (PASCAL, Topcon). Two weeks after arterial occlusion, the inner retinal layers (GCL-INL) disappear, while photoreceptors survive, albeit with edematous appearance of the ONL (Fig. 7). We explored the optimal extent of photocoagulation to achieve complete occlusion of the retinal branch artery, but without choroidal occlusion, so that photoreceptors are preserved. We also determined the optimal delay (2 weeks) between induction of the ischemia and retinal harvesting for integration of the photoreceptors with the host degenerate retina.

Finally, we assessed the physiological response of transplanted retina using visually Evoked Potentials (VEP) [30, 37, 38]. Using the Dome stimulation with Espion E2 electrophysiology recording system (Diagnosys LLC), we applied flashes of 4ms to both eyes simultaneously within 10-second inter-stimulus intervals. Even though, in some animals, we could observe the VEP in a transplanted eye, as shown in Figure 8, but we could not reproduce such success in every animal.

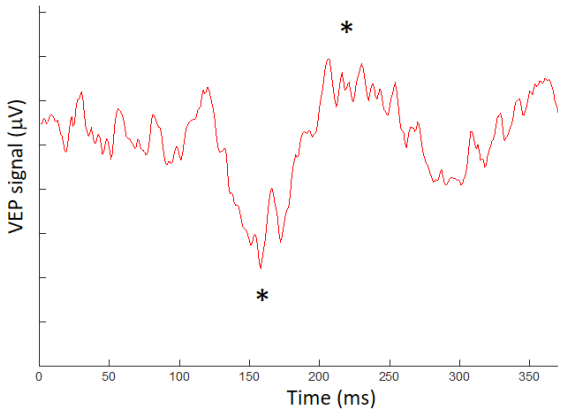

Figure 8: Visually evoked potential 5 months post transplantation. Dark adapted eye, white light in a dome, 0.5 cd·s/m^2 intensity. Prominent negative and positive waves indicated by the asterisk (*).

It is important to keep in mind that subretinal surgery in RCS rats has neurotrophic effect which extends survival of the photoreceptors[39]. Therefore, it is not enough to compare responses of the transplanted and non-operated eyes. To avoid any confusion with potential preservation of the native photoreceptors in the operated eye, we evaluated the residual VEP before photoreceptor transplantation. We can also used focal and pattern stimulation to reduce the non-specific VEP responses, if present.

Discussion

Transplantation of mature RPE/photoreceptors holds a high potential for rapid clinical implementation. First, human retinas can be harvested up to several hours post mortem and kept alive in culture for days [1] or even translocated within the same eye [40]. Second, the laminar organization of the retina allows relatively simple implantation of the sheet graft into subretinal space. Third, the immune privilege of the subretinal space [2-4] decreases the chance of rejection.

Several reasons could be behind the lack of reproducibility in previous clinical attempts of retinal transplantation. Some of them did not co-transplant RPE cells along with photoreceptors [41, 42], some only used retinal micro-aggregates with no preserved structure or orientation [12]. In the most convincing study, retinal/RPE sheets from unborn fetuses were transplanted into the central retina of several patients with retinitis pigmentosa (RP) and AMD [14]. Some of the patients showed an improvement of visual acuity in the transplanted eye, with the best increase from 20/800 to 20/200 over 5 years [14].

The extension of dendrites by the host bipolar cells in search of the new connections we showed here is in agreement with our previous observations that bipolar cells deafferented by photocoagulation extend dendrites toward the edges of the lesion and establish new functional connections with photoreceptors outside of the damage zone [22]. This suggests that reconnection is driven by bipolar cells rather than photoreceptors, and it likely involves secretion of proper cytokines by the mature photoreceptors, which is less likely to occur with underdeveloped stem cells.

Prior to our findings of better survival of the transplanted photoreceptors coupled to RPE, we tried a range of controls and other transplantation techniques which did not work. In particular, we observed no survival of the donor grafts having no RPE attached. We also tried retinal transplantation under the healthy retinas. However, the chronic retinal detachment created by the graft triggers degeneration of the host photoreceptors, accompanied by an inflammatory reaction, leading to elimination of the transplant as well.

While fetal retina has been documented to have higher stability to immune degradation in the subconjunctival space than adult full-thickness neuroretinal grafts [43], the use of mature donor tissue may offer multiple advantages. First, fetal retinas seem to develop into a 3-layered structure with a thick inner plexiform layer and RGC layer that could limit the establishment of

synaptic connections to the host [9]. Second, availability of the mature donor tissue eliminates an ethical and legal impasse. Finally, it opens the door to autologous transplantation in patients with central scotoma, such as geographic atrophy in AMD, with a technique similar to closure of macular hole [40]. Autologous transplantation in human patients would allow addressing some of the basic questions about photoreceptors survival and rewiring, without the confounding factors of histocompatibility. In addition, if removal of the inner retinal cells prior to transplantation would facilitate integration, that could be induced regionally by branch arterial occlusion with laser [36].

3. Summary

We demonstrated survival and integration of the mature photoreceptors transplanted with the retinal pigment epithelium (RPE). Full-thickness retina with attached RPE was harvested from healthy adult rats. Grafts were implanted into two rat models of retinal degeneration: Royal College of Surgeons (RCS) and S334ter-3. Survival of the host and transplanted retina was monitored using optical coherence tomography (OCT) for up to 6 months. The retinal structure and synaptogenesis between the host and transplant was assessed by histology and immunohistochemistry.

OCT and histology demonstrated a well-preserved photoreceptor layer with inner and outer segments, while the inner retinal layers of the transplant largely disappeared. Grafts including RPE survived better than without, and the transplanted RPE appeared as a monolayer integrated with the native one. Synaptogenesis was observed through sprouting of new dendrites from the host bipolar cells and synaptic connections forming with cells of the transplant. However, in many samples, a GFAP-positive membrane separated the host retina and the graft.

Presence of RPE in the graft improved the survival of transplanted photoreceptors. However, the lack of a reliable cortical response to stimulation indicates the very limited extent of the rewiring of the transplanted photoreceptors to the bipolar cells of the host. Functional integration between the transplant and the host retina might be enhanced if formation of a glial seal could be prevented.

4. Publications and presentations

Transplantation of Mature Photoreceptors in Rodents with Retinal Degeneration. H. Lorach, S. Kang, M. Bhuckory, A. Trouillet, R. Dalal, M. Marmor, D. Palanker. Translational Vision Science & Technology 8(3): 30 (2019).

Transplantation of mature photoreceptors in rodents with retinal degeneration. Daniel Palanker. Sixth Annual Retinal Cell and Gene Therapy Innovation Summit. Vancouver, May 2019.

Survival and integration of the retina/RPE allograft in rat models of retinal degeneration. D. Palanker, H. Lorach, S. Kang, A. Trouillet, R. Dalal. ISVER (Israeli Society for Vision and Eye Research) Annual Meeting, Ramat Gan, March 2018.

Long-term survival and integration of the retina/RPE allograft in rat models of retinal degeneration. H. Lorach, S. Kang, A. Trouillet, R. Dalal, D. Palanker. ARVO Annual Conference, Honolulu, April 2018. IOVS: 59(9):5000 (2018). ARVO abstract

Video 1. Transplantation procedure.

Video 2. 3D reconstruction of the transplanted area based on Bassoon (red), PKC (green) and DAPI (blue) staining. PKC indicates rod bipolar cells, Bassoon - presynaptic ribbon synapses, and DAPI outlines all nuclei.

5. Conclusions

Shear forces inflicted by explosion or head impact may result in traumatic retinopathy due to damage of the photoreceptors, leading to irreversible loss of sight. Retinal degeneration also leads to blindness due to gradual loss of photoreceptors. One potential strategy to restore visual function in such patients is replacement of the lost photoreceptors. We have discovered a very robust rewiring of the deafferented bipolar cells to photoreceptors migrating into the scotoma in animal models and in human patients. We also developed a method for extraction of the photoreceptors sheet from the donor retina and subretinal insertion of such monolayer of tissue.

In this study, we demonstrated survival and integration of the mature photoreceptors transplanted with the retinal pigment epithelium (RPE) in rat models of retinal degeneration. OCT and histology demonstrated a well-preserved photoreceptor layer with inner and outer segments, while the inner retinal layers of the transplant largely disappeared. Synaptogenesis was observed through sprouting of new dendrites from the host bipolar cells and synaptic connections forming with cells of the transplant. However, in many samples, a GFAP-positive membrane

separated the host retina and the graft. Most importantly, lack of reliable cortical response to stimulation indicates the very limited extent of the rewiring of the transplanted photoreceptors to the bipolar cells of the host. Functional integration between the transplant and the host retina might be enhanced if formation of a glial seal could be prevented.

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