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Retinal Transplants:

Hope to Preserve and Restore Vision

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Transplantation of retinal progenitor cell layers may be a viable treatment for devastating eye diseases such as retinitis pigmentosa and macular degeneration. In several studies of animals with retinal degeneration, transplants of intact sheets of immature retinal cells improved vision—a finding that was validated in recent clinical research in humans. However, data from more patients are needed to confirm this as a valid therapy.

The retina in the back of the eye contains two kinds of photoreceptor cells that are highly specialized for the detection of light—rods and cones. Rods are very sensitive to low levels of light (enabling vision at night) and to motion, and they are distributed evenly all over the retina, although they form a high-density ring around the macula, which is located in the center of the retina. Cones, on the other hand, are important for color vision in daylight; they are concentrated in the fovea in the center of the macula, which is responsible for sharp central vision.

Both rods and cones are supported by the retinal pigment epithelium (RPE), a cell layer that plays several important roles in the eye, including transporting nutrients from the blood vessel layer (the choroid) to the photoreceptors, eliminating their waste and forming the blood-retinal barrier that controls the transport of substances into the retina.

Retinal diseases such as macular degeneration and retinitis pigmentosa affect a sizable proportion of the world's population. In these diseases, photoreceptors and/or the RPE degenerate or become dysfunctional. However, the remaining inner neural retina and its connections to the brain can still remain functional—at least for some time—although the retinal neurons try to preserve themselves by rewiring, a process called "remodeling." If the diseased cells can be replaced and the new cells can connect appropriately with the still

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Retinal degeneration animal models

To study whether retinal transplants have an effect on vision, researchers must first transplant retinal tissue or cells into animals that have retinal degeneration, whether induced in the lab or as the result of a genetic defect. Most of these animal models have been developed in mice and rats, although some larger animals have also been used.

Light damage

This approach was used before genetic techniques for inducing retinal degeneration were available. The animals are continuously exposed to moderate light for several days or to short-term, intense light for 1-2 hours, which will lead to irreversible cell death (apoptosis) of rod photoreceptors. For example, exposing albino rats to two to four days of continuous blue light (680 to 1,290 lux) leads to a loss of rod photoreceptors but initially leaves the RPE intact.

Inherited RPE defect

A strain of rats with an inherited retinal degeneration was discovered in the late 1970s. Normally, rod photoreceptors receive all their nutrition from the RPE, which also digests the used-up outer segments of the photoreceptors. In rats with this defect, rod photoreceptors die because the non-functional RPE cells cannot digest the photoreceptor waste, leading to the accumulation of debris in the subretinal space that prevents nutrients from reaching the photoreceptors.

Research has shown that such rod photoreceptors can be "rescued" by RPE transplantation. They can also be transiently rescued by the injection of trophic factors, such as bFGF, or the transplantation of cells producing trophic factors. Although the rescued rod photoreceptors are not functional, saving them enables the rescue of cone photoreceptors, which depend on rods for their survival.

β-phosphodiesterase defect

Mutant mice with this well-known retinal deficiency have a defect in the gene encoding β-phosphodiesterase, an important enzyme in the process of transforming light into electrical signals. As a result, rod photoreceptors start to degenerate before their outer segments can form. Inner retinal cells respond to rod photoreceptor degeneration with retinal remodeling—in other words, the formation of aberrant synaptic circuits.

Transgenic models of retinal degeneration

There are now numerous lines of transgenic mice and rats with retinal degeneration that mimics human disease. They have been genetically manipulated to either express defective mutant human genes related to phototransduction (as can be found in retinitis pigmentosa) or in which the function of a phototransduction protein has been deleted. Some of these lines have been characterized and used to test various approaches of photoreceptor rescue. Our lab has transplanted retinal sheets in two different lines of rats carrying a mutant human rhodopsin gene.

functional part of the host retina, a degenerating retina might be repaired and eyesight restored.

People who are in the early stages of photoreceptor degeneration may be helped by taking vitamin supplements or trophic (growth) factors, or by undergoing transplantation of RPE cells or gene therapy to introduce trophic factors or correct mutated genes. However, once photoreceptors are lost, they must be replaced in order for vision to be restored.

There are three approaches to retinal transplantation. In the first and most common approach, RPE cells are transplanted to prevent continuing degeneration of the remaining rod photoreceptors. Alternatively, in the second technique, other cells can be transplanted that provide trophic factors to help maintain the cones, such as Schwann cells (supporting cells of the peripheral nervous system). The second approach is to replace the dysfunctional photoreceptors by new cells. A third strategy is to combine both RPE and retinal cells.

Our group has developed the third procedure, which is to implant sheets of fetal-derived RPE together with its neuroblastic retina. Currently, there are no realistic alternative therapies to retinal transplantation for end-stage disease, with the exception of microchip implantation ("retinal prosthesis"). In many retinal diseases, both the photoreceptors and RPE will be affected and both need to be replaced.

Transplantation of RPE cells to rescue photoreceptors

RPE cells and other cells delivering trophic factors can rescue photoreceptors in rats with defective RPE cells, but the animals still continue to undergo a very slow, progressive degeneration. Researchers have studied different aspects of functional restoration using such cell transplants (J. Leukoc. Biol. **74**, 151). However, clinical trials of RPE allografts in humans (tissue genetically non-identical or "mismatched" species) have failed because of tissue rejection.

Human clinical trials are ongoing with autologous transplants (the patient's own cells). These cells or tissues are taken from the healthy periphery of the patient's eye and placed under the damaged center of the eye; they are either injected as dissociated cells or translocated as patches of RPE and choroid, which is the blood vessel layer behind the RPE. The results of these studies have been mixed (Prog. Retin. Eye Res. **26**, 516).

However, photoreceptor rescue can be accomplished in other ways besides replacing dysfunctional RPE cells with functional ones. For example, treating patients with various growth factors, including basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF) and ciliary-derived neurotrophic factor (CNTF), can also rescue these cells. Currently, phase II clinical trials—which are designed to assess efficacy once a treatment's safety has been established in a Phase I trial—are under way with encapsulated RPE cells that produce the growth factor CNTF.

The best approach so far is to implant healthy immature cells that do not yet express surface antigens (which could trigger rejection) and that can develop into photoreceptors.

All of these approaches will only work in early stages of retinal degeneration, when there are still photoreceptors left to rescue.

What is the best donor tissue?

Although it makes intuitive sense to replace only the defective photoreceptors and not other cells, in reality this approach does not work. Photoreceptors are highly specialized cells that get destroyed if one tries to selectively replace them. In fact, Silverman and colleagues tried an approach to separate different retinal layers and transplant an isolated photoreceptor layer as a sheet, but the transplants became damaged, losing their outer segments and rolling up into balls.

However, photoreceptors can be maintained as a sheet if other parts of the retina are also included that contain supporting cells known as Müller glia. The downside here is that mature cells express surface antigens that can cause rejection if donor and recipient are not matched.

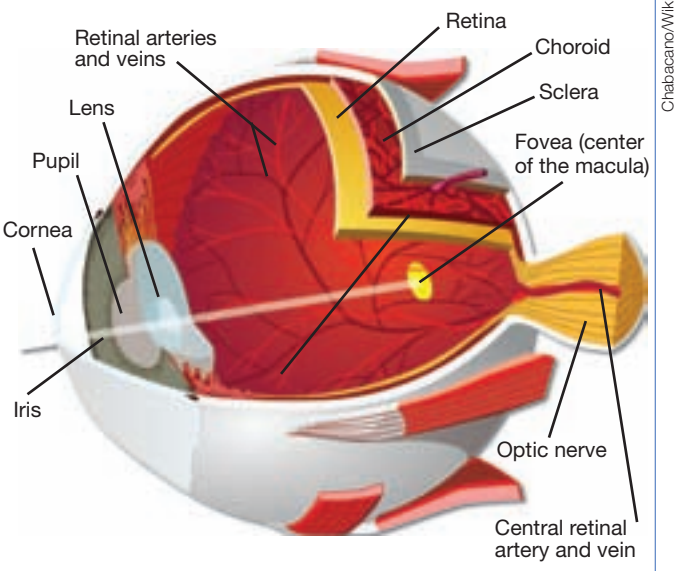
Thus, the best approach so far is to implant healthy immature cells that do not yet express surface antigens (which could trigger rejection) and that can develop into photoreceptors. Retinas derived from human fetuses contain neuroblastic cells that have the potential to develop into all retinal cell types. They are not rejected if they are transplanted to the same species within the central nervous system (of which the retina is a part).

Our group has developed a procedure to implant layers of freshly isolated neuroblastic fetal-derived retina, together with their supportive RPE, to the subretinal space. We started with studies of animals with retinal degeneration and eventually conducted research on humans.

To prepare the neural retina together with its RPE sheet, the tissue is treated with the enzyme dispase and thoroughly washed afterwards to be able to dissect the RPE cleanly from the choroid. The enzyme treatment is omitted for preparing the neuroblastic retinal sheet only. The dissected layers of donor tissue are maintained in cold "hibernation" medium with B-27 supplements until implantation.

Strict ethical procedures need to be followed when using fetal-derived tissue. Donors and recipients must sign informed consent forms that explain the purpose of the research and its potential risks. Even with numerous procedures in place, the use of discarded fetal-derived tissue is still controversial, and this tissue is difficult to obtain. Therefore, in the long term, it would be best to develop a renewable source of tissue by differentiating embryonic stem cells into implantable layers of retina and RPE cells.

[Diagram of the eye]



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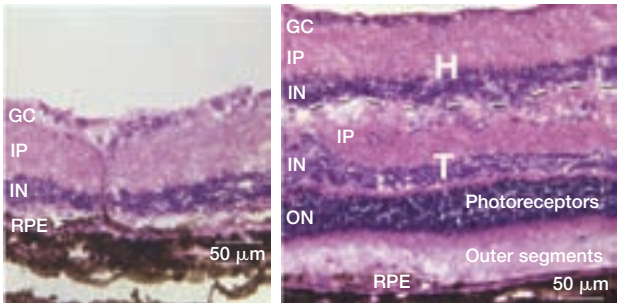
Light travels through the transparent cornea, and is focused by the lens (cut open in this diagram) onto the retina to the fovea (spot of focused vision).

[Before and after transplantation]



(Left) This degenerated rat retina has lost almost all of its photoreceptors, except for some residual cell bodies of cones. (Right) Retinal area restored by retinal transplant (T), 44 days after implantation. Most retinal layers have developed, including a large number of photoreceptors (dark blue stain) with extensions (outer segments). White dashed line indicates the border between transplant and host.

Abbreviations used in figures:

H = host, T= transplant	OP = outer plexiform layer
GC = ganglion cell layer	ON = outer nuclear layer
IP = inner plexiform layer	OS = outer segments
IN = inner nuclear layer	RPE = retinal pigment epithelium

This transplant patient from Louisville, Ky., U.S.A., can now see well enough to perform many tasks, including her hobby, painting ceramics. This picture was taken almost two years after surgery. Now, six years after the transplant, she has still mostly maintained her improved vision. She can work on her computer, read large letters, and has taken up sewing.

Pat McDonogh, ©2004 The Courier-Journal

Implantation procedure

Fetal-derived retinal tissue is very fragile, so it was necessary to develop an instrument and procedure for gently implanting intact sheets of the donor tissue and to test it on animals. The implantation instrument has a moveable flat Teflon nozzle. The nozzle is made in different sizes and curvatures depending on the size of the animal's eye, and on the surgical approach that is used.

The tissue layer is loaded in the flat nozzle tip so that polarity is maintained. Thus, the surgeon can be absolutely sure that it is not transplanted upside-down. Next, he or she places the donor tissue (no injection or pushing) with a very small amount of medium to the subretinal space in the eye, thus requiring only a small bleb to minimize trauma. The placement is entirely controlled by the surgeon.

Rodents have small eyes with a large lens; thus, the best surgical approach is to cut a small incision across the sclera (outer layer) of the eye. In larger eyes, such as those of cats and humans, a standard transvitreal surgical approach is used.

Identification of donor tissue within the host

In order to distinguish donor cells from host cells, the donor cells must contain a unique marker that is not found in the host, such as green fluorescent protein. For many years, our group has used transgenic rats that contain human placental alkaline phosphatase in all cells (Dev. Biol. **214**, 128). The implanted cells can divide, migrate and send out processes within the host and always be identified.

Optical coherence tomography (OCT) can be used to image transplants in the living eye, both in rats and in humans, to show the placement of the transplants in the subretinal space. The latest development of high-resolution OCT can even indicate how the transplants are layered.

Retinal layer transplants in animals

Only 20 to 30 percent of fetal-derived layer transplants in rodents will become successfully organized in a manner similar to a normal retina, with photoreceptor outer segments in contact with grafted or host RPE. The rest of the transplants will develop into rosettes (balls), sometimes with parallel inner retinal layers. Because of the small size of a rodent's eye, the surgeon cannot see where and how the donor sheet is placed. This makes the surgery very delicate. The challenge is to insert the nozzle in the right angle to place the tissue into the subretinal space without damaging the host RPE or the transplant.

There is a limited time window for successful implantation because the degenerating host retina and RPE seal together after a certain time, preventing insertion of a transplant into the subretinal space. In the optimal case, implanted layers of fetal-derived retina will develop photoreceptors with inner and outer segments when they are in contact with either the host or donor RPE. Optimally laminated transplants appear to establish a morphologically normal interaction with the host choroid of the recipient.

In contrast to transplants of fetal-derived retina, transplants of adult, fully developed photoreceptors maintain outer segments only if they are transplanted together with the adjoining part of the inner nuclear layer containing the Müller glia cells, which are needed to maintain the photoreceptors and act as a scaffold.

Retinal layer transplants restore visual function

By examining retinal transplants that were fixed in different stages of the light cycle of the transplanted rat, our research has indicated that, in laminated transplants, phototransduction proteins migrate inside the photoreceptors in response to light, resembling a normal function. However, it is very difficult to directly record electrophysiological information from the implanted retina to show visual responses.

Although several research groups—including those led by MacLaren and Radner—have shown host ganglion cell responses after retinal transplantation, investigators have questioned the validity of this testing procedure (Vision Res. **47**, 2815).

Retinal transplants have been shown to delay the deterioration of visual acuity in the implanted eye, using an improved optokinetic test that can analyze each eye separately.

The recording of global responses from inside the eye (via an electroretinogram) has limitations and is not sensitive enough for a small transplant in the eye. A more advanced, reliable method is to record from a visual brain center, the superior colliculus, to demonstrate visual responses. A very favorable anatomical feature of the superior colliculus is that the surface has a retino-tectal "map" corresponding to the different areas of the retina.

The restoration of visual responses has been shown in several retinal degeneration models, as described in the review by Seiler and Aramant. Responses were only found in a small area of the superior colliculus according to the placement of the transplant in the host eye. Restored transplant-specific responses can be recorded at relatively dim light levels (down to -3.4 log cd/m^2).

It is more challenging to demonstrate a transplant effect on visual behavior. Retinal transplants have been shown to delay the deterioration of visual acuity in the implanted eye, using an improved optokinetic test that can analyze each eye separately (J. Neurosci. Methods **138**, 7). In this test, rats are placed into the center of a rotating drum with vertical stripes. If the rat can see the stripes, it will automatically turn its head following the direction of the stripes of the rotating drum. Optokinetic tests have been used by other groups for a long time; however, those setups could only test both eyes at the same time and not separately (Vision Res. **40**, 2201).

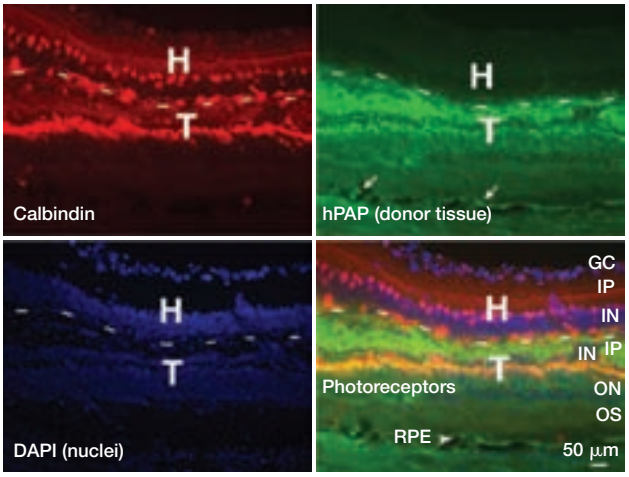
Retinal transplants connect with the recipient retina

Do transplant photoreceptors directly contribute to visual responses, or are the visual responses due to a more unspecific "trophic" effect of the transplant on some residual host cones? To answer this question, researchers have used trans-synaptic tracing with a special "neurotropic" (i.e., directed against neurons) pseudorabies virus, which is specifically transferred between neurons at synapses. Although glial cells can take it up, they cannot transfer it to other cells.

After this virus is injected into the superior colliculus at the point that is estimated to correspond to the transplant placement in the retina, it is transported back to the ganglion cells of the host retina. From these host cells, the virus is transferred to host interneurons in the inner nuclear layer and then to cells in the transplant.

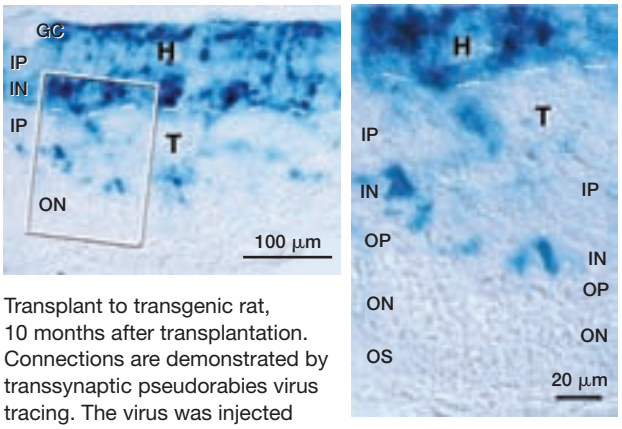
In more recent experiments, virus tracing was performed after electrophysiological recordings. Again, the virus labeled the host retina over the transplant and neuronal cells in the transplant. These studies indicated synaptic connections between transplant and host.

[Cell types and integration of graft and host]



A fetal-derived retina was transplanted together with its RPE to a rat with an inherited retinal degeneration. The border between transplant and host is indicated by a white dashed line. Note the good integration. (Top left) Horizontal and amacrine cells stained by calbindin antibody (red). (Top right) Identification of donor cells by label for human placental alkaline phosphatase (hPAP) (green). The arrows point to labeled donor RPE cells. (Bottom left) Nuclei of all cells stained blue with DAPI (4′6-diamidino-2-phenylindole dihydrochloride). (Bottom right) Merged image. Transplant 167 days after implantation.

[Demonstration of graft-host connections]



Transplant to transgenic rat, 10 months after transplantation. Connections are demonstrated by transsynaptic pseudorabies virus tracing. The virus was injected into a visual brain center, the superior colliculus. Three days after the injection, the virus (blue label) has heavily labeled the host retina overlaying the transplant, and has also labeled cells in the transplant. Since the virus can only be transferred from one nerve cell to the other, this indicates that there are synaptic connections of host with graft cells. (Left) overview; (bottom) enlargement.

The FDA eventually allowed transplantation to patients who had vision of 20/800 or worse in one eye, and then later to those with 20/400 and 20/200 vision.

Finally, we have used the donor cell label called human placental alkaline phosphatase (hPAP) to look at the transplant-host interaction on the electron microscope level. Donor cells and processes can be identified by immunohistochemistry or histochemistry. This study is still in progress, but preliminary results indicate the presence of many donor-derived processes in the host retina.

Clinical trials

Our preclinical experiments have shown that human tissue can be transplanted to special rats with a defective immune system, preventing rejection of the foreign tissue. In the subretinal space of this rat, fetal-derived human retina with its RPE can successfully develop retinal layers with photoreceptors in contact with the co-transplanted RPE; this apparently "normal" organization of the human transplant was maintained after more than 10 months.

Such studies laid the groundwork for clinical trials in humans. After the procedure was approved by the U.S. Food and Drug Administration, its safety was established in 5 retinitis pigmentosa patients with varying levels of light perception. Although the procedure seemed to be safe, there was no evidence that it was effective in these patients: No vision improvement could be measured by Early Treatment Diabetic Retinopathy Study visual acuity testing.

The FDA eventually allowed transplantation to patients who had vision of 20/800 or worse in one eye, and then later to those with 20/400 and 20/200 vision. Between 2002 and 2006, a total of 10 additional patients (six patients with retinitis pigmentosa and four with dry age-related macular degeneration) received sheet transplants of fetal-derived retina together with its RPE (review in Aramant et al., *Retinal Degenerations: Genetics, Progression, and Therapeutics*).

Seven of these patients showed improvements in visual acuity. One remained unchanged, while the vision of two patients continued to deteriorate. Patients were tested three times pre-operatively and at least seven times after surgery. The best patient improved from 20/800 pre-operatively to 20/160 at one year post-op. Her vision in the eye that underwent surgery maintained its improvement—it was 20/200 at more than five years post-op.

No clinical evidence of rejection was observed, although the transplant sheet lost its pigmentation by six months. Besides the objective visual improvements, patients also reported subjective improvements in their quality of life, which mostly correlated with the objectively tested visual acuity.

Retinal transplantation has shown promising results both in animal models and human patients. The research gives hope for a viable therapy to help people with retinal diseases.

Future directions

Several research groups are working to develop neural retina and RPE from embryonic stem cells. This would provide an unlimited supply of donor tissue. Our hypothesis is that the stem cells can be developed into three-dimensional layers before transplantation and implanted in a similar way as described for fresh fetal-derived tissue. This will likely require an extensive research effort to accomplish.

Magdalene Seiler and Robert Aramant have a proprietary interest in the implantation instrument and procedure. Their current work is supported by the Lincy Foundation, NIH R44 EY015584; and private donations.

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