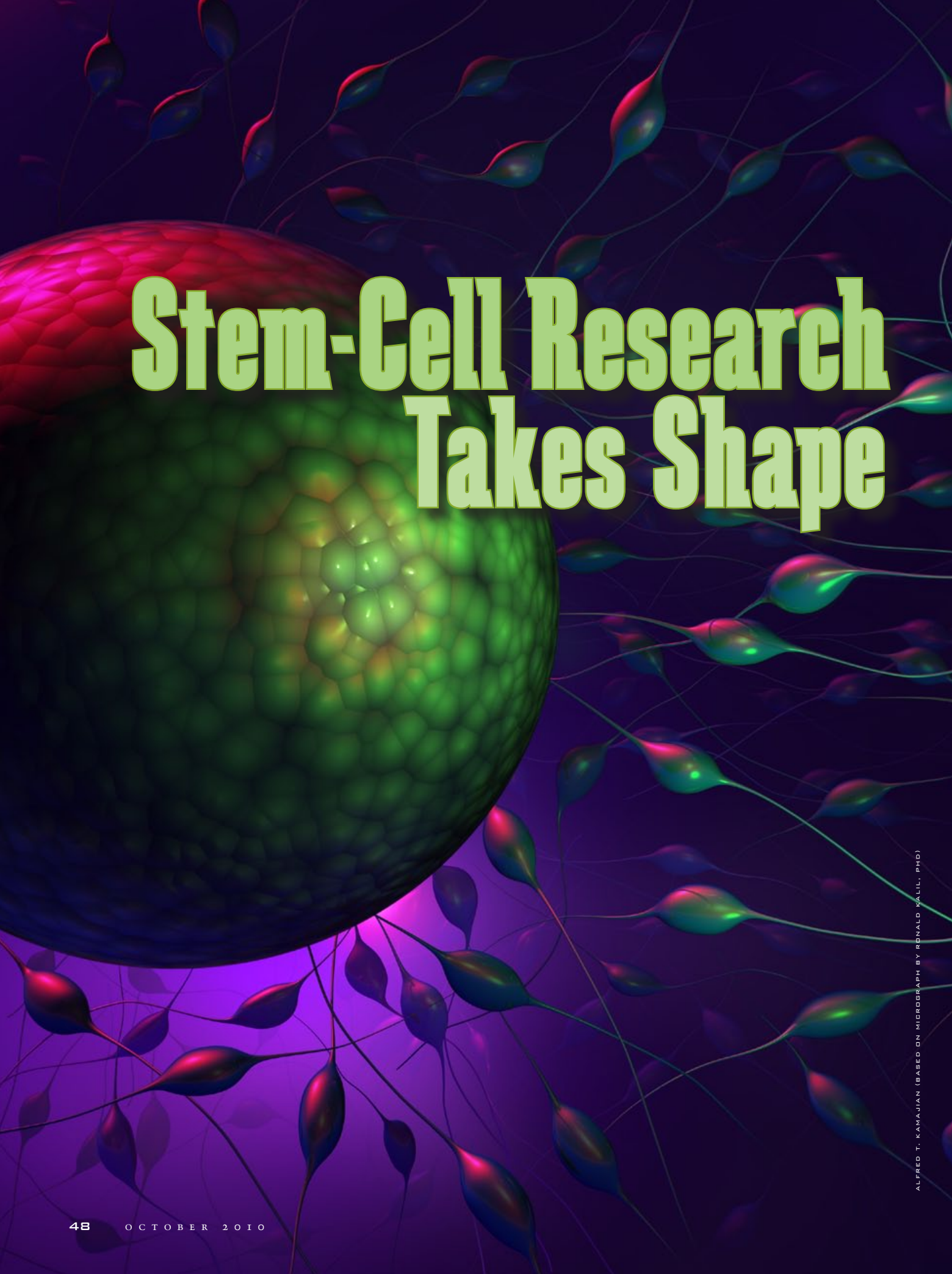
Stem-Cell Research Takes Shape



ALFRED T. KAMAJIAN (BASED ON MICROGRAPH BY RONALD KALIL, PHD)

Despite their microscopic size, stem cells are very big newsmakers. Their capacity to become any other cell type powers the miraculous engine of complex life. That capacity, in fact, may drive the future treatments of choice for many diseases.

By Annie Stuart, Contributing Writer

In terms both literal and figurative, the eye provides a very good lens through which to assess stem-cell science. The visibility of the eye's structures makes them far more open to this assessment than anatomical sites like the heart, gut or brain, where keeping tabs on transplanted cells and averting tragedies is no small matter.

And as a visible extension of the brain, the retina's circuitry is already well-known and better understood than some other areas of the nervous system. "For stem-cell applications to central nervous system diseases, the eye will likely lead the way," said David M. Gamm, MD, PhD, assistant professor of ophthalmology and visual sciences at the University of Wisconsin in Madison. "It's accessible, it's amenable to manipulation, and you can observe donor and host cells in real time. And the techniques and instrumentation needed to introduce cells into the eye are readily available."

There are also advantages of focusing on eye diseases from a study safety standpoint, said Dr. Gamm. "You have two eyes and they are encapsulated organs, so if something were to go wrong, you could more easily contain the problem."

Given the eye's research strengths, some believe the fate of stem-cell medicine rests with ophthalmology. Here is a survey of this rapidly evolving field.

A TALE OF THREE CELL TYPES

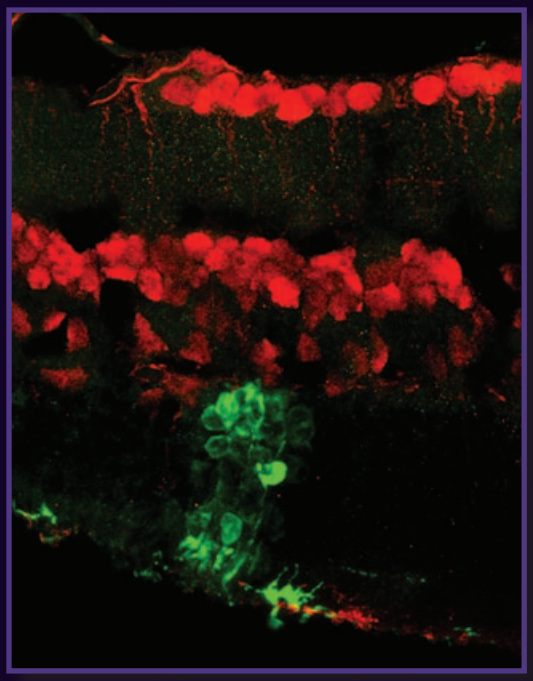
The first stem cells to be studied as possible therapeutic weapons against human disease were the true originals—human embryonic stem (hES) cells. But stem-like cells can actually be obtained from differentiated tissue. These are induced pluripotent stem (iPS) cells and regeneration-capable adult (tissue-stem) cells.

Just when researchers thought they had mastered the intricacies of the hES cell, the iPS cell came along through a technique pioneered in 2006 by researchers at the University of Kyoto.[1] This technique reprogrammed already differentiated adult mouse skin cells back to an undifferentiated state that mimicked many of the hES cells' properties. Over time, it was found that reactivating just four dormant genes could induce pluripotency in these adult cells, said Deepak A. Lamba, MBBS, PhD, research assistant professor of ophthalmology at the University of Washington in Seattle. Many labs today are experimenting with both hES and iPS cells, and some have retrieved tissue-stem cells from the eye, as well.

But these three cell lines have epigenetic differences. And they can act differently in different settings, said Dr. Lamba. So exactly how useful each will become is up in the air. "It all comes down to the field maturing," he said. Following are particulars of each cell line.

1. Human embryonic stem cells. With their original, all-purpose potential, hES cells are relatively efficient and consistent. "The stem-cell community has a lot of collective experience with the common hES cell lines. In essence, we are using the same lump of clay," said Dr. Gamm. But the use of a human blastocyst from which the cells are retrieved remains an ethical conundrum for some. And the cells pose concerns for tumorigenesis and immunological problems from mismatches between donors and recipients; these concerns won't be resolved until the cells make their way through clinical trials.

2. Induced pluripotent stem cells. "iPS cells appear to be a dramatic breakthrough and in many ways they are," said Ronald E. Kalil, PhD, professor of ophthalmology and visual sciences and of neuroscience at the University of Wisconsin in Madison. They're "almost" pluripotent but not quite, he said. "And, initially iPS cells were capable of creating tumors when transplanted into animals because at least one of the transcription factors used to reprogram fibroblasts was an oncogene. So over the last 18 months, there's been an intense degree of activity to try to eliminate harmful transcription factors. And this culminated about a year ago in reprogramming fibroblasts just by putting proteins into them. This eliminated interference with the host cell's genome." However, this also introduced a new problem: low yield.



Integrated human embryonic stem-cell-derived photoreceptors in green show characteristic morphology following subretinal transplantation in mice. In red are Pax6+ host horizontal, amacrine and ganglion cells.

So the next effort will be to increase the efficiency of production of reprogrammed cells, said Dr. Kalil.

Along with these issues are problems of inconsistency, said Dr. Gamm. "With iPS cells, there are a lot of different lines and everyone is handling them differently." iPS cell lines derived from the same fibroblasts using identical procedures can differ from each other. "Some lines may be relatively easy to differentiate into certain cell types," said Dr. Kalil, "while others are recalcitrant. The process of reprogramming has a number of variables that stem-cell scientists don't yet fully understand."

iPS cells may solve the immunological issues associated with hES cells, but even that's not entirely worked out, said Peter J. Coffey, PhD, head of ocular biology and therapeutics at University College London Institute of Ophthalmology in the United Kingdom. Dr. Coffey explained that the process of reprogramming or correcting genetic faults could possibly alter immunological markers or introduce a protein not recognized by a person's immune system.

Though the challenges may seem daunting, iPS research is light years ahead of where things with hES cells were a few years after their discovery, said Dr. Gamm. "So, it's just a matter of some catch-up and continued effort to understand how these cells are made and how they behave on a very basic stem-cell biology level."

3. Tissue-stem cells. Some differentiated cells can seemingly remember how to behave like their progenitor stem cells. "If you look at really small animals like salamanders, you can essentially scoop out the retina and it will form a new retina," said Dr. Lamba. "Unfortunately, as humans evolved, we lost a lot of our regenerative capacity. However, a few researchers have found a few human cells here and there that have some regenerative capacity, but it is extremely limited."

Some scientists first considered the ciliary margin to be a potential source of retinal stem cells, but that now appears not to be the case, said Dr. Coffey. "People are still looking at the retina and components in the retina that may at least have the potential to generate some type of retinal precursor."

The cornea, on the other hand, has a clearly identified source of stem cells, and they elicit fewer problems like tumorigenecity and immune rejection than do hES and iPS cells. "There's been great success there for the last 10 years using limbal corneal stem cells to repopulate the ocular surface after chemical burns and for certain genetic disorders," said Dr. Coffey.

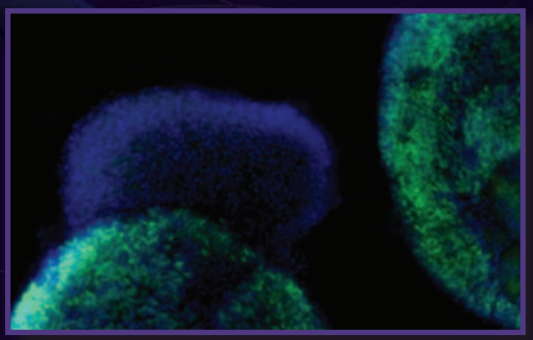
A GOAL WITH THREE STRATEGIES

With the overarching goal of exploiting stem cells to eliminate eye disease, ophthalmic researchers have generally chosen to work with three strategies:

1. Model disease in a dish. Because iPS cells are created from simple biopsies of already differentiated cells, they provide a ready tool for studying pathogenesis and potential bioassays for therapeutics in those specific cell types, said Dr. Coffey. "Until now, we had never had access to the diseased tissue at a quality and level that would allow us to do many of the experiments we would like to do."

Dr. Gamm cautions, however, that care will obviously need to be taken with iPS cells. "Many diseases require precise interactions between cell types. Different organs may even be involved, which may have to play off one another to produce a disease phenotype. So there are levels of complexity that you can't recapitulate at this point in a dish."

Still, he agreed, the potential is great. "Everyone is talking about replacing cells and doing transplants, but rushing to do so could set the whole process back tremendously. There are a number of very useful things that can be done even without ever putting a cell into a



Two populations of cells derived from human embryonic cells; forebrain progenitors are in blue and retinal progenitors in green.

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person," he said. "I think the tortoise is going to win here and not the hare."

2. If replacing diseased cells, keep it simple. Where replacement is the goal, the simpler the cell and the simpler the connection, the better, said Dr. Gamm, adding that this is a strong argument for first targeting the outer retina and the retinal pigment epithelium, as opposed to the inner retina. "Replacing a ganglion cell for glaucoma or optic nerve diseases is an even higher level of difficulty, requiring replacement of a cell that has a strict correspondence with a distant region of the central nervous system. To begin, we're looking to replace a single cell type in a relatively simple, well-described disease where all the other puzzle pieces in the retina are in place and functioning well."

Photoreceptors may pose too big a challenge at first, said Dr. Coffey. "Trying to replace a cell as specialized as a photoreceptor and then to get it to reconnect within a neural structure, which is the retina itself, is very complex as opposed to trying to replace a single layer of support cells, such as the RPE."

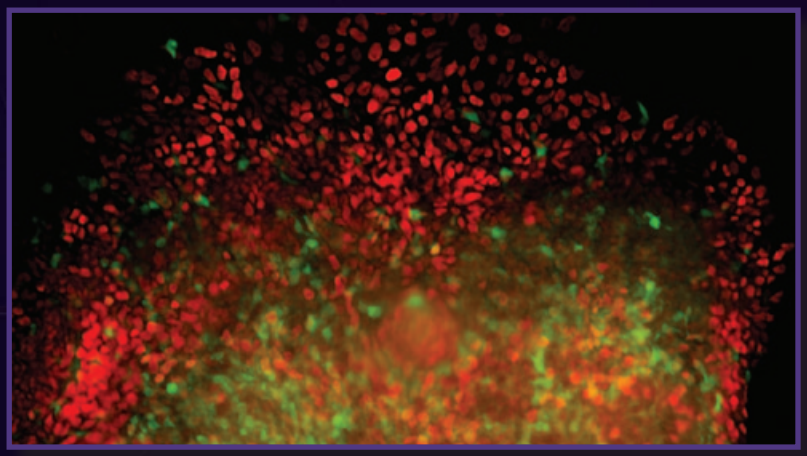
Timing is crucial, too, said Dr. Gamm, because a lot of diseases in the outer retina produce a domino effect over time. "It might be a disease centered in the RPE that eventually causes the photoreceptors to die, followed by inner retinal circuitry changes, gliosis and scarring. Each domino that falls increases the complexity—perhaps exponentially—of trying to develop a therapy."

3. Or don't replace cells—rescue them. Many scientists believe that stem-cell therapy will make its biggest impact, not by replacing diseased cells but by rescuing them.[2] "Stem cells have a number of properties that may help keep dying host cells alive," said Dr. Gamm. "They tend to survive well in the subretinal space and can be genetically modified to secrete growth factors, like mini drug factories. They also migrate well and can integrate within host tissues where they can play a supportive role." All of this also makes it difficult sometimes to tell exactly what a transplanted stem cell has accomplished—whether they are frankly replacing cells or simply reviving dysfunctional cells, he said.

Linking stem cells with the products of engineered cells may offer a customized combination for producing miniature drug factories with local delivery, especially for diseases with disseminated effects. "Many injured or diseased cells may be dying because they're not getting proper life support from a growth or neurotrophic factor," said Dr. Kalil. Transplanting engineered cells near the degenerating tissue may slow intrinsic cell death. And, because many stem cells naturally produce growth factors, genetic modification might not even be necessary, he said.

TWO EYE SITES OF RESEARCH

Cornea out in front. Providing both easy access and well-defined stem cells at the narrow annulus circumscribing it, the cornea allowed stem-cell researchers a jump start in this field. These limbal stem cells are a focus of research for both Nick Di Girolamo, PhD, associate professor of pathology and director of ocular research at the University of New South Wales in Sydney, Australia, and Francisco C. Figueiredo, MD, PhD, of the North East England Stem Cell Institute in Newcastle upon Tyne in the United Kingdom.



Fluorescence image showing human embryonic stem-cell-derived retinal cultures differentiating to photoreceptor cells (red) and amacrine and ganglion cells (green).

After limbal stem-cell biopsy—with ex vivo expansion in the laboratory and subsequent transplantation—was pioneered in 1997 in Italy, Dr. Figueiredo and collaborators set a goal to improve on the technique for patients with total unilateral limbal stem-cell deficiency caused by chemical or thermal burns. "We wanted to do something that would pose less risk to the patient," he said. "At the time, what was used was a technique for skin stem cells. For this technique, you had to use animal cells and products to expand them in vitro, putting them in a culture where they could grow but still retain their stem-cell properties." The researchers' goal was to emulate the technique but develop an animal-free method, one that used a human tissue substrate—amniotic membrane from the placenta—full of growth factors for facilitating stem-cell growth.[3] Up to four years after transplantation, corneal stem cells are still functioning in all patients,

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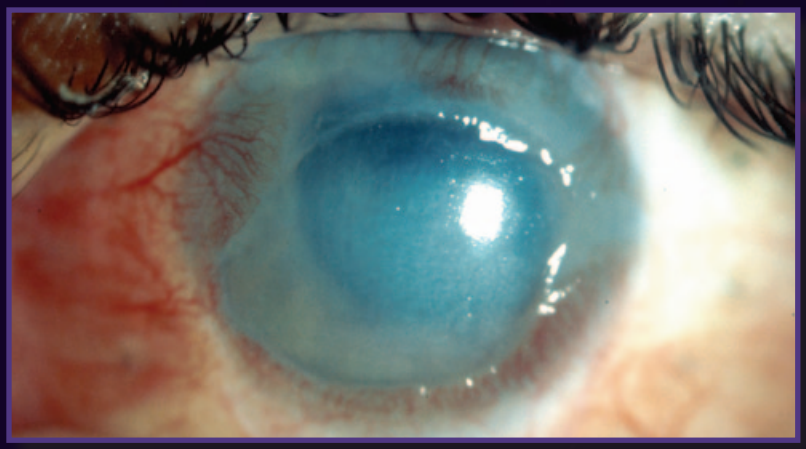


Patient with total unilateral limbal stem-cell deficiency caused by chemical injury warranting limbal stem-cell transplantation. New vessels in the cornea, red eye, a hazy cornea, and a large central corneal epithelial defect are all typical signs of limbal stem-cell deficiency, along with very poor sight and constant pain.

producing greatly improved quality of life by reducing pain, glare and photophobia but also improving patients' vision.

In another variation of this autologous approach, Dr. Di Girolamo and colleagues have harvested limbal stem cells from patients' healthy eyes and placed them on an FDA-approved hydrogel contact lens, where the cells are nurtured in the patients' own serum. These are patients with unilateral limbal stem-cell deficiency due to chemical or thermal burns, multiple surgeries to the ocular surface, or aniridia.[4]

Although a small percentage of the cells that migrate from the tissue biopsy are stem cells, said Dr. Di Girolamo, the rest are daughter cells with a high proliferative capacity, and they rapidly cover the contact lens surface over two to three weeks. In a cellular version of "Red Rover, Red Rover," the cells "jump" from the contact lens polymer to the human ocular surface. "We're not quite sure how this happens," he said. But with this procedure his team has been able to create a stable, transparent corneal epithelium, partially restoring vision and correcting problems such as large painful ulcers and conjunctival pannus.

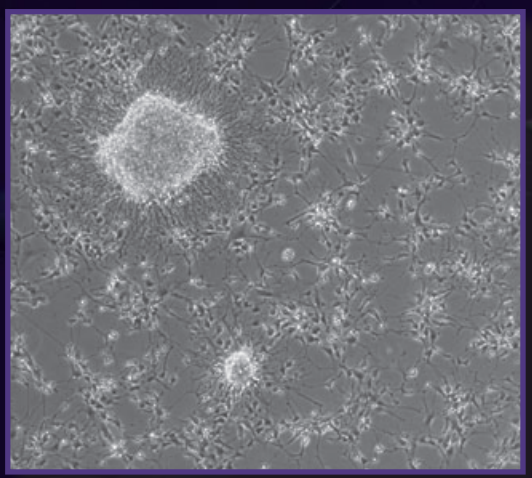
Momentum for the corneal stem-cell therapy is growing. A recent Italian study involving 112 patients with corneal burns reported "permanent restoration" of clear epithelium in 76.6 percent of eyes by treating them with autologous limbal stem cells that had been cultivated on fibrin.[5]

Retina brings up the rear. Dr. Gamm's lab is focusing on the forks in the road that determine early cell fate decisions for the retina: "What makes an undifferentiated pluripotent human stem cell become virtually any cell type in the body? What makes it decide to be a neural cell, then a neural cell in the anterior portion of the brain? And later on, a cell belonging to the retina, and, finally, a photoreceptor or RPE cell? Right now, it's still a black box."

Every step in cell differentiation builds off preceding steps, so understanding early stages is essential, Dr. Gamm said. "What we've done is to develop a protocol whereby we can derive retinal cells in a step-by-step manner from hES and iPS cells." This system allows his lab to follow the development of these cells from an undifferentiated state all the way through the production of cells that express markers of mature photoreceptors, revealing a pathway that closely mimics what's expected in the normal course of human retinal development. Other labs have published similar protocols, he said, but with the like-minded goal of working forward from the most basic scientific principles to achieve efficient production of cell types with therapeutic value.

Dr. Lamba's group was among the first in the United States to take undifferentiated hES and iPS cells and very efficiently transform them into retinal cells.[6] "We have been characterizing these cells since 2006 to figure out if we have any kind of contamination." In 2009, his team was able to show that these light-sensing cells could restore some vision in a mouse model of Leber congenital amaurosis.[7]

Remarkably, the visual improvement was comparable to restoring enough vision for a completely blind person to navigate across a room, said Dr. Lamba. "We feel it's efficacious but want to make sure that it will not be deleterious to the patient in any way," he said, explaining that the present goal is to thoroughly



Micrograph of hESC-derived neural stem cells viewed with a phase contrast microscope. The globular shapes are neurospheres—colonies of tightly packed stem cells—surrounded by individual cells that are migrating away.

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characterize the cells, ensuring that they won't form tumors. "We've never seen it, and I think it is because our protocol is a very efficient way of making the cells; but we need to make sure we know everything we have in our dish before we put them in people."

Referring to the 2009 paper, Dr. Kalil lauded the work of Dr. Lamba and his collaborators. "The transplanted cells integrated with the photoreceptor layer and behaved like photoreceptors," he said, noting that the cells displayed electrical activity reminiscent of an eye's normal response. "It's one of the best papers in the field showing that the cells have some potential, at least in respect to photoreceptor degeneration, to do something productive after they're transplanted into adults. And, that's the $64,000 question: You can produce cells that replace cells, but are they going to integrate with the circuitry?"

In 2006, Dr. Coffey's lab announced the audacious goal of taking stem-cell therapy into the clinic for macular degeneration within five years. "There is good clinical evidence to suggest that as long as you can get RPE back under the macula, you can stop someone from going blind," he said, emphasizing that timing is also key. "We developed a human embryonic stem-cell line that gave good differentiation of retinal pigment epithelium and could be produced in quantity. And we've manufactured membranes of cells so we can grow these on patches and hope to place those patches surgically." Dr. Coffey's team is in preclinical stages. He said they are now 18 months away from a phase 1 clinical trial using patches of RPE in 10 patients. Dr. Coffey is optimistic this approach will be successful, in part because his team has not yet seen an immunological response to the cells in animals.

Also moving to clinical trials is the company Advanced Cell Technology, which hopes to use stem-cell therapy to replace RPE cells in patients with Stargardt macular dystrophy.

THE PLURIPOTENT FUTURE

Although the hope is that these studies will shed light on efficacy, they may only show safety, said Dr. Coffey. And, said Dr. Kalil, a single gold standard may remain elusive since different approaches will likely be used for different problems, providing something more akin to a stem-cell tool kit. "One of the beauties of this field is that it proceeds like an old motor vehicle, chugging along and then suddenly jumping forward. And who can predict when that will happen? There are extraordinarily talented people working on this, and we'll likely see this come to fruition within the next 10 years."

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MEET THE EXPERTS

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