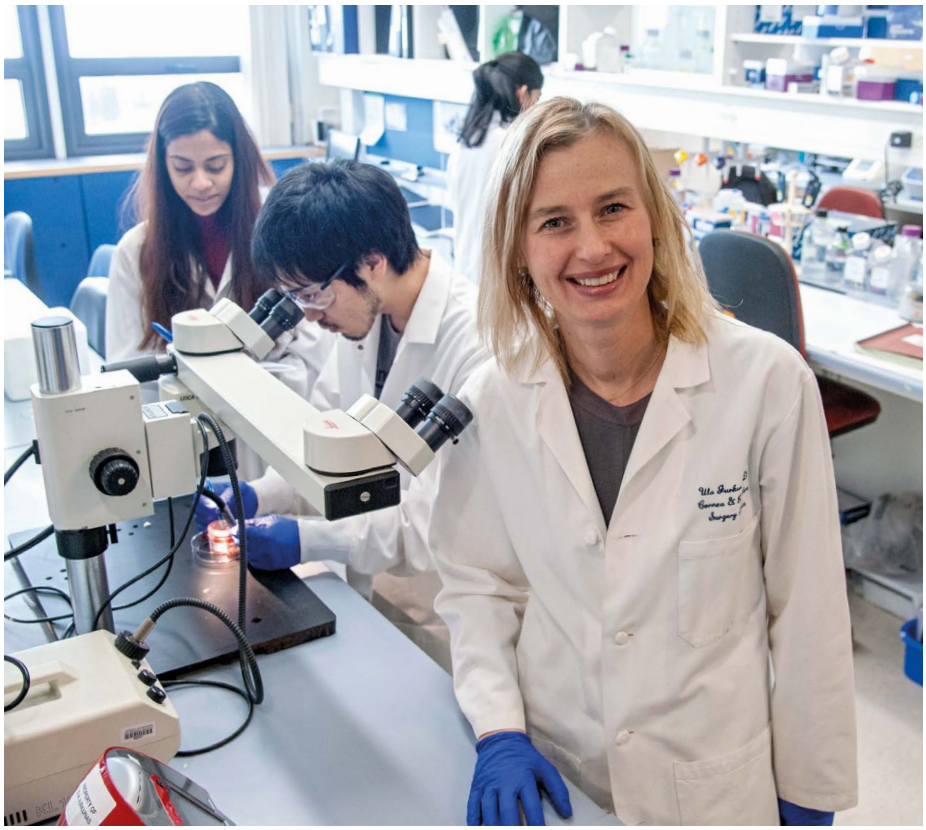


Ula Jurkunas (front) is an eye surgeon at Massachusetts Eye and Ear in Boston.

JOHN EARLE PHOTOGRAPHY FOR MASSACHUSETTS EYE AND EAR

A new vision for stem cells

Regenerative therapies for the eyes could help to reverse several causes of blindness. **By Neil Savage**

Nick Kharufeh celebrated US Independence Day last year with his family, by setting off fireworks outside his aunt's home in Rialto, California. A large rocket, instead of flying skywards, hit the ground and exploded, shooting a burning fragment into his face and immediately blinding him in his left eye. At the hospital, doctors told him his eyelid, cornea and lens had been burnt beyond saving, and that he was not a good candidate for a corneal transplant. With that news, the 23-year-old began to face the prospect of living with just one eye, and tried to accept that his dream of becoming a commercial pilot was probably over.

One year on, Kharufeh's eyesight is on the way to returning to what he hopes will be something close to normal, thanks in part to an experimental stem-cell treatment. He is one of 13 participants in an early-stage clinical trial at Massachusetts Eye and Ear, a hospital and research centre in Boston, that is using stem cells from an individual's healthy eye to repair their damaged cornea.

Fireworks are a common cause of injuries to the cornea, says Ula Jurkunas, an eye surgeon who is leading the research. But there are plenty of other ways in which this transparent cover on the front of the eye can be damaged. "It could be infections and it could be congenital disease," she says. In low- and middle-income countries, a leading cause of corneal blindness is trachoma, an infectious disease caused by a bacterium.

The eye is fertile ground for stem-cell therapies. It is surgically accessible, and physicians can easily monitor how well treatments are working. Jurkunas's study is one of several taking place worldwide that use stem cells to repair damaged corneas. Other researchers are trying to regenerate cells in the retina — the main light-sensing part of the eye — to treat other common causes of blindness such as age-related macular degeneration and glaucoma. Although these therapies will not reverse all cases of blindness, they could go a long way towards restoring some vision to many people.

Window repair

In a healthy eye, the cornea is surrounded by the limbus, a structure that contains corneal epithelial stem cells. These cells give rise to an epithelial layer that protects and supports the cornea (see 'Inside the cornea'). But injury or infection can deplete those stem cells, taking away an otherwise healthy cornea's capacity to heal and causing it to turn opaque, blocking vision.

Damaged corneas can be replaced, but without stem cells to replenish the epithelium, the transplant will deteriorate in a similar way over time. "Full corneal transplant just replaces the central 8 millimetres," Jurkunas says. "If you don't have those stem cells in the periphery, that transplant will not live."

Jurkunas's approach to rectifying this problem starts with her taking a small sample of stem cells from a patient's healthy eye and giving it to Jerome Ritz, an oncologist at the Dana-Farber Cancer Institute in Boston who specializes in culturing cells. Ritz grows the cells on a sample of amniotic membrane, a layer of the placenta that has long been used as a graft to promote healing in damaged corneas. Once a big enough sheet of stem cells has been created, Jurkunas transplants them into the damaged eye.

In some people whose stem cells have been depleted but who have an otherwise undamaged cornea, that patch is enough to restore sight. Other people, such as Kharufeh, need a conventional corneal transplant from a donor. Kharufeh has also had to have his eyelid reconstructed, and is due to have an artificial lens implanted in October.

Jurkunas and Ritz have been developing this method of cultivating and expanding stem cells since 2006. To avoid potential contamination, they grow the stem cells in a sterile laboratory where workers wear protective suits and nurture the cells without using antibiotics or hormones. This strict protocol allows them to meet US Food and Drug Administration (FDA) guidelines for producing pure products that don't carry infections or have other unintended effects.

A commercially available corneal epithelial stem-cell product, Holoclar, uses cells from mice as a feeder layer to support stem-cell proliferation[1]. Holoclar was developed in Italy

by scientists at the University of Modena and Reggio Emilia in Modena and at the pharmaceutical company Chiesi, based in Parma. Jurkunas and Ritz, by contrast, have opted not to use mouse cells – a decision based in part on the belief that regulators would worry that animal cells could transmit animal diseases to humans.

In 2015, the European Medicines Agency approved Holoclar for use in the European Union; in the United States, it has so far been approved only for experimental use. Jurkunas says the use of feeder cells complicates the process of getting FDA approval for a therapy, and her version that does without them might have an easier time. But it will take further clinical trials, and probably the interest of an industry partner, before Jurkunas and Ritz's technology is submitted for approval.

One possible path towards commercialization would be to grow stem cells in volume, instead of transplanting them from a patient's good eye each time. That would make transplants available more quickly and open up the procedure to people who have damage to both eyes. It is an approach being taken by Kohji Nishida, an ophthalmologist at Osaka University in Japan, who has grown sheets of corneal epithelial cells from scratch using induced pluripotent stem cells (iPS cells) – cells harvested from a donor and coaxed into becoming first stem cells, then a specific tissue.

"If you don't have those stem cells in the periphery, that transplant will not live."

Scientists have experimented with creating everything from retinas to heart muscle from both iPS cells and stem cells taken from donated embryos, but hadn't had much luck with corneal epithelial cells, Nishida says, in part because the eye consists of so many different types of cell. His solution is to create an eye organoid – a 3D structure that grows in a lab dish and mimics the development of a whole eye – from which the epithelium, itself containing some stem cells, can be taken and transplanted[2]. "We expect that because the iPS-cell-derived corneal epithelial cell sheet contains enough corneal epithelial stem cells, vision restoration should be maintained for many years – hopefully forever," he says.

Nishida has transplanted the sheets into four people. None of those transplants was rejected, and all four recipients experienced some improvement in their vision, Nishida says. He expects to wrap up the first phase of his clinical trial by March 2022, after which he hopes to expand the trial to several sites and obtain approval for a clinical treatment within a few years.

In a potentially less invasive approach to repairing the corneal epithelium, Che Connon, a tissue engineer at Newcastle University, UK, is studying how the stiffness of tissue underlying epithelial stem cells affects their growth. He discovered that the substrate in the limbal area, where the stem cells grow most easily, is softer than in the centre of the cornea, and that corneas injured by a chemical burn can become stiffer. That stiffness somehow prevents the stem cells from proliferating. In testing on rabbits, he found that by treating the affected area with an enzyme that digests collagen and temporarily softens the tissue, he could encourage the stem cells to grow new layers[3]. "We could get a repair not by doing a limbal stem cell transplantation," Connon says, "but actually just by restoring the home environment."

Connon has also been trying to grow entire corneas that would eliminate the need for donors, by introducing stem cells into a 3D printing process. He mixes stem cells with collagen and an extract from algae to create a bio-ink, which he deposits in concentric circles on a curved substrate to build a cornea[4]. He expects it will take several years to perfect the process, by which point it could help people who otherwise can't get transplants. "We can quite easily imagine a future," he says, in which the "lack of donor tissue is no longer a problem".

Into the retina

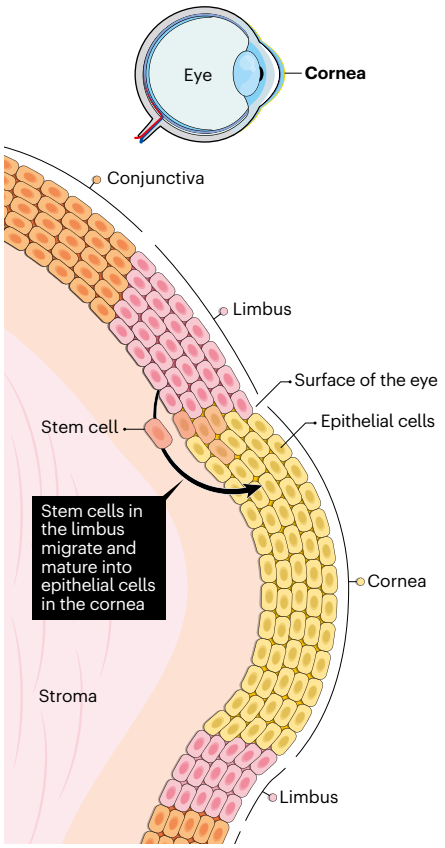
Cornea damage is only one cause of blindness; problems deeper in the eye are both more common and more challenging to address. The leading source of blindness in people aged over 60 is age-related macular degeneration, a condition affecting almost 200 million people worldwide, in which the central portion of the retina begins to degrade. This is the result of a breakdown in the retinal pigment epithelium (RPE), a layer of cells that absorbs light and supports the photoreceptor cells that transmit signals the brain can interpret as images. When RPE cells degrade, the photoreceptors die off and the signal pathway is cut, shutting down sight. Problems in the RPE are also associated with other visual impairments, included retinitis pigmentosa, which causes night blindness and tunnel vision, and diabetic retinopathy, which can lead to people with diabetes losing their sight.

During the first decade of this century, researchers tried to grow RPE cells from stem cells to replace those that were lost. They didn't have much success. Part of the problem was that those early attempts simply injected the new cells into the eye, says Kapil Bharti, a cell biologist at the US National Eye Institute in Bethesda, Maryland. RPE cells work together as a layer of tissue, and injecting them doesn't seem to encourage them to organize themselves, he says. By 2013, researchers were finding that the cells worked better when implanted as a solid layer, an approach that Bharti follows. He grows the RPE cells on a biodegradable polymer scaffold already approved by the FDA, and transplants them as a single sheet.

To produce the RPE cells, Bharti uses iPS cells derived from the individual's own blood cells. The advantage of this approach over donor stem cells is that such patient-derived transplants don't require the use of immunosuppressive drugs to prevent the body from rejecting the transplanted tissue. "These patients are usually in their eighties and they don't tolerate these strong immune suppressants," he says. Additionally, to ensure the transplanted cells won't lead to cancerous growth or other issues, researchers test them to check that they're genomically stable and pure. They look for cancer-promoting genes

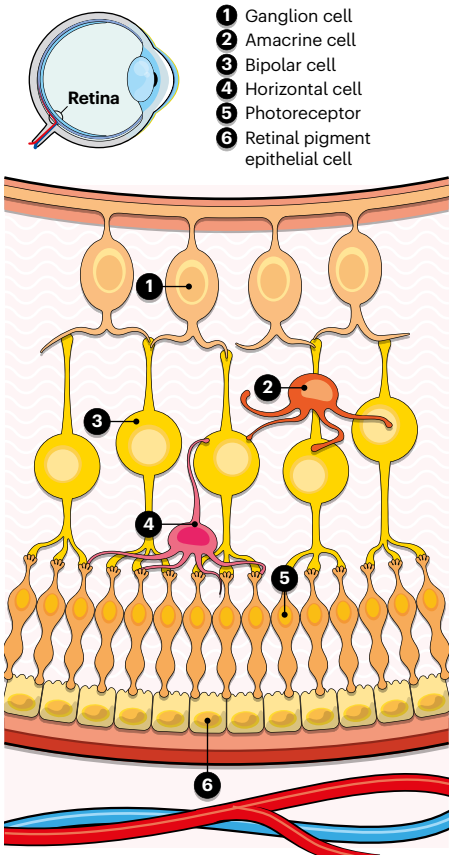
INSIDE THE CORNEA

The cornea is surrounded by several cell types, including stem cells that give it the capacity to heal.



WIRING THE RETINA

The retina is composed of several cell types that collect light and transmit signals to the brain.



and remove any cells that test positive, and they also check that there are no stem cells left in the RPE tissue to be transplanted, to ensure that it won't keep growing beyond what's needed.

Bharti is running an early-stage phase I/IIa clinical trial, which he says is the first in the United States to study transplants grown from participants' own iPS cells. He will transplant his RPE sheets into a dozen individuals and assess the safety of the procedure, checking to see whether the transplanted tissue stays alive and doesn't cause any problems over five years. All of the participants have advanced disease and have therefore also lost photoreceptors and other cells, so he doesn't expect any improvement in their vision. "I'm hoping that it will halt the disease progression at the stage when it's transplanted," he says. "I don't think it'll reverse much of what is lost."

To explore the possibility of restoring some lost vision, Bharti is working with David Gamm, an ophthalmologist who directs the McPherson Eye Research Institute at the University of Wisconsin–Madison. Gamm is using stem cells to grow photoreceptors — the cones that are responsible for colour vision and the rods that allow sight in low light.

Although growing RPE cells can be challenging, they're the soul of simplicity compared with photoreceptors, which Gamm says are among the most complex and specially structured sets of cells in the body. "Photoreceptors don't grow as a single monolayer of purified cells," he says. "They grow within a neural retinal structure. So you essentially have to grow the entire neural retina in order to make a developmentally correct, authentic photoreceptor cell." It takes months to grow a retina, which will contain cones sensitive to blue, red and green light, as well as rods.

Gamm has founded a Madison-based company, Opsis Therapeutics, that has been developing a way to create photoreceptor cells at a commercial scale. It started out in 2015 with a manual approach, growing organoids containing various cell types and then picking out the photoreceptors. For the past four years it has worked with Fujifilm Cellular Dynamics, a stem-cell company in Madison, to develop a process to make enough of the cells for a commercial therapy.

Reaching for the brain

The photoreceptor cells attach to the RPE layer on one side and, on the other, to bipolar cells, which in turn link to retinal ganglion cells that relay electrical signals from the retina to the brain along the optic nerve (see 'Wiring the retina'). Damage to the ganglion cells occurs in glaucoma, a blindness-inducing disorder often caused by increased pressure in the eye.

Because ganglion cells tie directly into the brain through nerve fibres called axons that are several millimetres long, they are especially challenging to regenerate, says Petr Baranov, an ophthalmologist at Massachusetts Eye and Ear and Harvard Medical School in Boston. In 2019, Baranov led a team of researchers at Harvard and the Moscow Institute of Physics and Technology that transplanted retinal ganglion cells grown from stem cells into mice. They used iPS cells and embryonic stem cells from mice to grow a retinal organoid, then selected out the ganglia, put them into solution, and injected them into mice whose own ganglion cells had been killed off.

About 65% of the transplanted ganglion cells integrated themselves into the retina and survived for at least 12 months — a substantial portion of a mouse's lifetime[5]. The researchers were also able to measure signals travelling from the retina to the brain, showing that the new ganglia were functional. They have not yet tested whether the transplants affect the mice's vision, but Baranov hopes to do that in the next year or two. For now, they're working with small numbers of cells so they can track them individually and figure out how well they're performing. "We transplant 10,000 to 20,000 cells per eye," he says. "To recover vision, you probably need hundreds of thousands of cells."

"What we hope to do is make a meaningful change in a patient's visual function."

Once researchers have learned enough about how to build and integrate ganglion cells to try the procedure on humans, Baranov hopes that they might be able to restore vision to people who have lost it to glaucoma. "I don't see the limitations, because what we know is that the brain and the retina, the remaining neurons, they remain elastic enough to allow them to form new connections," he says. The team also artificially increased pressure in the eye in mice to simulate a disease state, and the donor cells survived. That stress test provides hope that transplanted ganglia might be able to function normally in diseased eyes.

But fully restoring eyesight remains a dream. Some treatments for certain conditions go a long way towards improving vision, and, as scientists learn to regrow and replace different components of the eye, those various pieces might come together to restore some of what's been lost.

Gamm cautions against expecting too much, too soon. "It's unreasonable to think that we are going to cure blindness in general, just like we're not going to cure cancer with one magic bullet," he says. "What we hope to do is make a meaningful change in a patient's visual function, such that their activities of daily life are improved."

As with any technology, stem-cell therapies for eyesight will be expensive and limited in their capabilities to begin with, and will continue to get better over the years, Gamm says. But improvements in vision don't have to be huge to make a difference in people's lives. "Patients who undergo degeneration of their vision will tell you, if only I could be like I was a year ago, I would be fine," Gamm says.

Neil Savage is a freelance writer in Lowell, Massachusetts.

1. Rama, P. *et al. N. Engl. J. Med.* **363**, 147–155 (2010).
2. Hayashi, R. *et al. Nature* **531**, 376–380 (2016).
3. Gouveia, R. M. & Connon, C. J. *Trans. Vis. Sci. Tech.* **9**, 5 (2020).
4. Isaacson, A., Swioklo, S. & Connon, C. J. *Exp. Eye Res.* **173**, 188–193 (2018).
5. Oswald, J., Kegeles, E., Minelli, T., Volchkov, P. & Baranov, P. *Mol. Ther.* **21**, 180–198 (2021).

Correction
This Spotlight article misspelt Kharufeh's name throughout.