News in Review

A LOOK AT TODAY'S IDEAS AND TRENDS

Stem Cells in Mice Hold Promise for RP

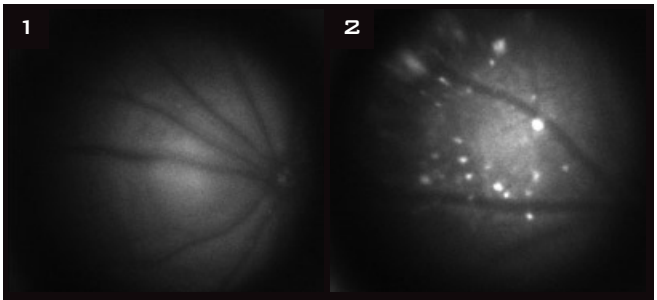
Led by researchers at Columbia University, an international team may have moved stem cell transplantation one step closer to an ophthalmic application by restoring visual function in a mouse model of retinitis pigmentosa.[1] A first of its kind, the seven-month study achieved restoration of sight in 25 percent of mice receiving reprogrammed embryonic stem cells, although 50 percent of all test mice developed benign tumors or retinal detachments.

The researchers derived the RPE-like cells from closely related mice, a strategy with similarities to induction of pluripotency using a skin biopsy from the patient, said lead author Stephen Tsang, MD, PhD, assistant professor of ophthalmology, pathology and cell biology at Columbia University Medical Center in New York City. Using congenic or induced pluripotent stem cells circumvents the problem of rejection, which requires immunosuppression and has plagued cell-based therapies in the past.

After differentiation began, the researchers implanted the yellow fluorescent protein–labeled stem cells in the subretinal space and closely watched their fate over time using noninvasive fundus autofluorescence imaging. "We were able to monitor the stem cells and actually track the one-to-one correlation of the dots on the retina," said Dr. Tsang. In addition, the researchers measured the retina's response to light using electroretinography.

"The transplanted cells not only looked like retinal cells, they functioned like them, too," said Dr. Tsang. "Because the retina is part of the brain, you will not get function back if the retinal cells don't talk to the rest of the neurons." Luckily, it is easier to monitor survival and efficacy of stem cell grafts here than in the rest of the brain, and it's only necessary to manage output since input is provided by light.

In this study, only differentiated stem cells were shown to "produce rescue," or restore function—not undifferentiated stem cells, fibroblasts or feeder cells. The source of rescue in other rodent models has been more difficult to determine, said Dr. Tsang. For example, with the Royal College of Surgeons rat model, morphological rescue can be explained, in part, by disruption and washing of debris in the subretinal space seen



STEM CELLS. A control mouse eye (1) and an eye with stem cells (2). After differentiation into RPE-like structures, embryonic stem cells were labeled with a yellow fluorescent protein. Using fundus autofluorescence imaging, the researchers were able to see the cells (bright spots) in the mouse retinas.

EyeNet *thanks Susan B. Bressler, MD, for her help with this issue's* News in Review.

in rats up to six months after sham injections.[2]

Despite their success in restoring function, Dr. Tsang and colleagues are now faced with complications of tumors and the resulting retinal detachments. They are currently testing stem cells newly engineered with a herpes thymidine kinase suicide gene to ablate tumor development. "Because the eye is a transparent organ, we can see the cells and know whether they've developed tumors," he said. "Then we can deliver ganciclovir to eliminate them." (Ganciclovir targets the stem cell that's been engineered with the herpes suicide gene, and this gene is not expressed unless a tumor develops.)

Another challenge of the study was the researchers' inability to determine the total number of cells remaining in the subretinal space. Dr. Tsang said the researchers are now addressing this by using autofluorescence imaging with a much wider angle, which makes it possible to count more cells after surgery.

Although the study was aborted at seven months while the facility was upgraded to pathogen-free status, the researchers have restarted the experiment to see if it can carry on for the life of the mouse, said Dr. Tsang. Once complications are resolved, the hope is the technique might be used for a wide range of retinal diseases because it is based on a rodent model that is generalizable to many human forms of retinal degeneration.—*Annie Stuart*

1 Wang, N. K. et al. *Transplantation*. Published online Feb. 15, 2010.
2 Li, L. and J. E. Turner. *Exp Eye Res* 1991;52(6):669–679.

Retina Report

Nanotech May Offer Solution for Gene Therapy

Instead of using a virus' genetic machinery to insert protective genes into degenerating retinas, perhaps the DNA could be hidden inside nanoscale molecules capable of sneaking into the nuclei of nondividing cells, a recent study by Tufts University researchers suggests.[1]

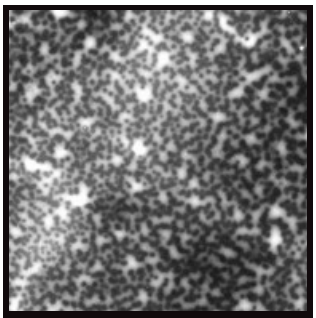
The scientists report that they succeeded at inserting plasmid DNA into mouse retinal pigment epithelial cells by enclosing it inside peptide nanostructures about 136 nm across. Built from linked molecules of a peptide called POD ("peptide for ocular delivery"), the nanoparticles had been modified with polyethylene glycol (PEG), in a process known as pegylation.

Mouse eyes injected with PEG-POD nanoparticles showed 215 times the level of gene expression of eyes injected with DNA alone, the researchers report. Nanoparticles made from two other PEG-modified peptides produced 56.52- and 24.73-fold enhancements in gene activity.

"This is one of only a few studies demonstrating that a nonviral vector is capable of delivering genes to the retina in animals," said study leader Rajendra Kumar-Singh, PhD, associate professor of ophthalmology and neuroscience at Tufts. "Many nonviral vectors for gene therapy have been developed, but few, if any, work in postmitotic tissues such as the retina."

Nonviral gene delivery is viewed as a possible way to address concerns about inflammation and serious or fatal immune reactions to viral vectors being used for gene therapy.

Currently, synthesized nanostructures are used to encapsulate and transport large, therapeutic molecules, such as drugs, to targeted sites in the body and protect them from degradation. The size, shape and surface characteristics can be customized and precisely controlled in the laboratory.



A CLOSE LOOK. An electron micrograph of the PEG-POD DNA complexes indicates that each nanoparticle is approximately 136 nm in size.

To carry plasmid DNA, Dr. Kumar-Singh and colleagues designed a synthetic peptide based on a binding domain of basic and acidic fibroblast growth factor. Multiple copies of the POD peptide can be linked together to make PEG-POD-DNA nanoparticles.

Pegylation was essential to compacting the plasmid DNA and keeping the POD nanoparticles small and separate from one another, Dr. Kumar-Singh said. PEG prevented the negatively charged DNA and the positively charged POD molecules from forming a cross-linked aggregate too big to pass through the intact nuclear membrane.

Nanoparticle-delivered genes would need to function longer than they did in the current study (one week) in order for this approach to be viable for a disease such as retinitis pigmentosa or age-related macular degeneration, Dr. Kumar-Singh said. Follow-up studies will look at this issue.

The level of protein produced in the PEG-POD-DNA-treated eyes has been shown elsewhere to be sufficient to rescue photoreceptors in animal models of eye diseases.

"The number of cells that were transfected in our experiment is actually quite small. A few transduced cells were responsible for the majority of the signal we detected," he said. "We would like to deliver the gene to a much larger number of retinal cells." —*Linda Roach*

1 Read, S. P. et al. *J Gene Med* 2010;12(1):8.

Systemic Drug Update

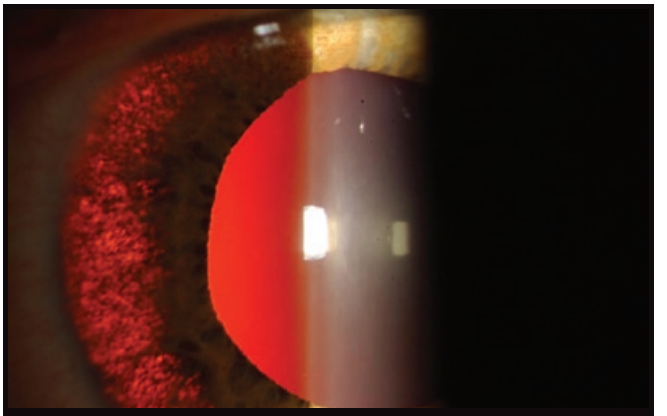
Oral Moxifloxacin May Cause Uveitis-Like Signs

Ophthalmologists in the Netherlands and Belgium saw uveitis-like episodes, iris transillumination, sphincter paralysis, persistent mydriasis and photophobia after oral moxifloxacin use in five patients. Marijke Wefers Bettink-Remeijer, MD, and associates at the Rotterdam Eye Hospital, the University Hospital Brussels and the University Hospital Ghent report the adverse effects in *Eye*.[1] Dr. Wefers Bettink-Remeijer is head of the emergency and accidents department and is a subspecialist in neuro-ophthalmology and ultrasound at the Rotterdam Eye Hospital.

Moxifloxacin is a synthetic broad-spectrum antibiotic often prescribed for the treatment of community-acquired pneumonia and acute episodes of chronic bronchitis and bacterial sinusitis. Few studies have addressed the ophthalmic effects of the oral use of the drug.

"One of the reasons for the adverse side effects could be the tissue affinity of moxifloxacin," said Dr. Wefers Bettink-Remeijer. "My guess is that the concentration in the iris is higher after oral use than after local use."

The five patients experienced adverse effects 10 to 14 days after use of oral moxifloxacin. All patients had used the recommended dose of one tablet for five to 10 days. After the patients were treated with topical prednisone, uveitis symptoms resolved and changed into permanent diffuse iris translucency and dilation. Pigment could no longer be detected. Visual acuity, however, remained unaltered. There was no elevation of the intraocular pressure.



AFTEREFFECT. Iris transillumination in a patient who took oral moxifloxacin.

The researchers note that the high tissue affinity of moxifloxacin could induce phototoxicity in the iris pigment, but they say that is not likely the only cause. They conclude that there may be multiple factors leading to the adverse effects, and they are convinced that use of moxifloxacin is one of those factors.

—Arthur Stone

1 Wefers Bettink-Remeijer, M. et al. *Eye* 2009;23(12):2260–2262.

Nutrition News

Green Tea: It May Be Good for Eye Health

Green tea has been touted for its ability to prevent everything from diabetes to stroke, and it could also have a protective effect against eye disease.

Now researchers at The Chinese University of Hong Kong, Hong Kong Eye Hospital, have reported that antioxidative substances in green tea, known as catechins, penetrated the eye tissues of rats that were orally fed a green tea extract.[1]

This study is built upon earlier studies showing a relationship between oxidative stress and cataract formation, ganglion cell death, and damage to retinal tissue and trabecular meshwork cells. The authors theorized that green tea consumption could benefit the eye against oxidative stress, if the green tea catechins could penetrate into eye tissues.

The researchers dissected the rat eyes into cornea, lens, retina, choroid-sclera, vitreous humor and aqueous humor. They found that catechins, particularly gallocatechin and epigallocatechin, were selectively absorbed into the different eye components.

Maximum concentration of the protective catechins occurred in the retina, followed by the sclera, lens and finally the cornea. The concentrations were higher in vitreous than in aqueous humor. In some eye tissues the green tea extract reduced oxidative stress for up to 20 hours. The authors say their findings suggest that drinking green tea could provide effective antioxidant activity in the retina, but less so in the cornea.

While other antioxidative substances have been tested in the eye (such as vitamin C, vitamin E, lutein and zeaxanthin), the authors claim this is the first evaluation of green tea's antioxidative effect in the mammalian eye.

They note that green tea has been consumed safely for thousands of years. Now, they write, "Our results indicate that green tea consumption could benefit the eye against oxidative stress."

—Miriam Karmel

1 Chu, K. O. et al. *J Agric Food Chem* 2010;58(3):1523–1534.

EYE 2009;23(12):2260–2262