Zapping hearing loss

By Lev Osherovich, Senior Writer

The most advanced cochlear implants for hearing loss lack frequency resolution and dynamic range, causing recipients to perceive sounds as loud, monotonous and robotic. An Australian team may have solved those problems with a combination product that uses electric fields generated by a cochlear implant to electroporate a gene therapy into the cells of damaged inner ears.[1]

Hearing device maker **Cochlear Ltd.** has an option to license the technology.

Profound hearing loss can be caused by congenital absence of—or damage to—hair cells in the cochlea, a spiral structure in the inner ear that decodes sound waves into neurological impulses.

Patients with total hearing loss can benefit from cochlear implants, which are multielectrode arrays inserted into the cochlea. The implants encode sound into electrical impulses that mimic the activation of hair cells.

However, replacing hair cell function with an electrical implant solves only half of the problem in patients who lose their hearing later in life. The other half is the loss of trophic factors that promote or maintain neuronal connections to the ear.

Ordinarily, trophic factors such as brain-derived neurotrophic factor (BDNF) are produced by mesenchymal cells in the inner ear and stimulate the growth of neuronal connections. In damaged ears, the production of BDNF is limited, causing nearby neurons to atrophy and disconnect from the cochlear lining.

Thus, restoring trophic factor production in the inner ear has been a major goal for enhancing the performance and useful lifespan of cochlear implants.

"The idea of delivering neurotrophins to the inner ear has been around for a while. The hope has been that this would increase survival of neurons," said Allen Ryan, a professor of surgery at the **University of California, San Diego**.

Current methods to deliver trophic factors include osmotic pumps, which act as extended-release reservoirs, or gene therapy using viral vectors. Ryan said that these approaches have yielded few benefits in animal models of hearing loss.

"People put in an osmotic pump and they see growth of neurites, which are small projections from neuronal bodies that connect to adjacent cells and develop into synapses," said Ryan. "The problem with pumps is that they eventually run out of the trophic factor, and then the neurites die."

"Getting genes into the ear is tricky," continued Ryan. "Doing this in such a way as to avoid an immunological response, and delivering enough of the viral vector, is a challenge."

Now, a team from **The University of New South Wales** led by professor of neurology and neuromuscular diseases Gary Housley has solved the problem of how to deliver trophic factors to the inner ear.

The team adapted electroporation—a well-known technique for introducing foreign material into cells using a brief burst of high-voltage electricity—to deliver a plasmid encoding *BDNF* into specific portions of the cochlea that are in contact with a cochlear implant.

"What we have done is targeted delivery adjacent to the neural interface," said Housley. "This creates a gradient of BDNF that stimulates regeneration of the neural processes" that enable hearing.

Housley's technique improves on previous trophic factor delivery strategies and provides a high degree of spatial control over where the transgene goes.

Genetic engin-ear-ing

Housley's team began by modifying the electrical parameters of an off-the-shelf device from Cochlear to provide brief but powerful electrical pulses at specific spots along an eight-electrode array inserted into the cochlear spiral (*see* **Figure 1, "Gene therapy by cochlear implant"**).

Housley's team inserted the modified implant into the ears of deaf guinea pigs and then bathed the cochlea with a solution containing a plasmid encoding human *BDNF* along with a GFP reporter.

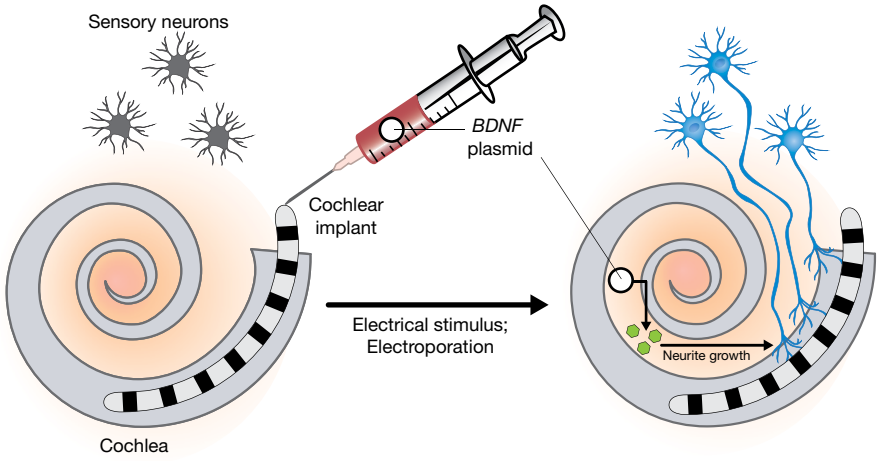

Figure 1. Gene therapy by cochlear implant. Cochlear implants are multielectrode arrays that treat severe forms of hearing loss caused by the absence of mechanosensory hair cells. Pinyon et al. adapted a cochlear implant marketed by **Cochlear Ltd.** to deliver a plasmid encoding *brain-derived neurotrophic factor* (*BDNF*), a growth factor that promotes sensory neuron growth. Activation of selected electrodes along the implanted array led to localized plasmid delivery to cochlear tissue near the activated electrodes. BDNF production and neurite outgrowth occurred near the regions that received plasmid but not in other parts of the cochlea.

Activation of individual electrodes along the implant led to electroporation of the plasmid into nearby mesenchymal tissue. After two weeks, nearby cells expressed both the *BDNF* and *GFP* transgenes.

The transgenic BDNF produced by transfected mesenchymal cells promoted the growth of neurites from nearby spiral ganglion neurons toward the electroporated cells.

BDNF transgene expression led to better implant performance than no transgene expression. In an electrophysiological assay of implant responsiveness, animals with implants that received the *BDNF* transgene showed a lower threshold for activation—and thus greater sensitivity—than animals with conventional implants.

The improved implant performance "reflects a graded, progressive increase in the number of nerve fibers," said Housley. "We've expanded the dynamic range of the nerve response."

Results were reported in *Science Translational Medicine* and are covered by patents filed by **NewSouth Innovations Pty. Ltd.**, the technology transfer arm of the university.

Cochlear contributed materials and expertise to the academic team's work through an **Australian Research Council**–sponsored Linkage Project, a translational R&D scheme that helps Australian companies and researchers collaborate on preclinical studies.

Hear today, gone tomorrow?

M. Charles Liberman, a professor of otology and laryngology at **Harvard Medical School**, said that the study is the first practical demonstration of localized gene therapy using electrical stimulation. "This is the first time that integration of electroporation into bionic interfaces—in this case the cochlear implant—has been demonstrated," he said.

Liberman added that lowering the threshold of activation for cochlear implants could open paths to miniaturizing cochlear implants, which currently include bulky power supplies that sit behind the patients' outer ears.

"They show that they can reduce the electrical threshold for excitation needed to stimulate fibers in the vicinity, leading to higher sensitivity," he said. "Lower threshold reduces power consumption, which gets you closer to devices without external power supplies."

Housley also suspects that electroporation of *BDNF* could improve the ability of patients to distinguish signals from each electrode, leading to better frequency resolution in existing devices. Alternatively, the technology could be used to increase the number of electrodes and thus the number of distinguishable frequencies in next-generation devices.

"As it is, today's implants have up to 30 electrodes, but in effect you get only 6–8 channels," said Ryan. This is because the relatively high electronic currents needed to activate each electrode tend to spread to adjacent regions of the cochlea and limit the resolution of sound frequencies.

The lower activation thresholds made possible by stronger local neuronal connections "mean there's an opportunity to use much lower stimulus levels," said Housley. "This also increases the number of electrodes we could use without current spread."

Housley and Cochlear SVP and CSO Jim Patrick cautioned that the technology needs considerable preclinical work. One major question is whether the effects of the *BDNF* transgene expression will be long lasting.

"This work is just starting," said Patrick. "In their paper, Housley's team showed good short-term improvement, but longer-term effects would be needed for this to be clinically useful."

Along these lines, Ryan said that a next step would be constructing a more robust gene expression vector than the plasmid used by Housley's team. He suggested repeating the experiment with a permanent gene-editing technique such as clustered, regularly interspaced short palindromic repeats (CRISPR).

Another question is whether BDNF is the best trophic factor for the job. Housley said that his team started with BDNF because it is a known entity. Liberman thinks a different growth factor—neurotrophin 3 (NTF3)—may have a more potent effect on neurite regeneration than BDNF.

"Our data show that in the cochlea, NTF3 is really more relevant than BDNF," said Liberman. "If you did this with NTF3, you would get more bang for the buck."

Housley said that experiments with *NTF3* transgene electroporation are under way.

Liberman also wanted to know whether *BDNF* transgenes could improve hearing function throughout the entire cochlea and not just in the accessible portion studied by Housley.

Housley said that his team has yet to test hearing function of treated animals using behavioral assays, so it is unclear how the improved electrophysiological functioning his team observed affects perception of sound.

Patrick noted that the company is interested in adjunct therapies to enhance the performance of its products.

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