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Gene therapy boosts the bionic ear

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Close-field electroporation gene delivery using the cochlear implant electrode array enhances the bionic ear (Pinyon *et al.*, this issue)

Hearing loss is a major health and economic burden on society. The World Health Organisation estimates that over 5% of the world's population – 360 million people - suffer from a *disabling* hearing loss (*1*). Hearing impairment can result in significant communication disorders, leading to important educational, social and vocational ramifications that can adversely affect quality of life and is associated with dementia in the elderly. Sensorineural hearing loss is the most common form of deafness and typically occurs following the loss of cochlear hair cells that normally convert acoustic vibrations into nerve impulses within the primary cochlear afferents or spiral ganglion neurons (SGNs; Fig. 1) (*2*). Patients with a severe-to-profound sensorineural hearing loss receive no benefit from amplification via a conventional hearing aid due to an absence of hair cells; the only therapeutic intervention available to these patients is a cochlear implant (*3*).

Cochlear implants, or “bionic ears”, electrically stimulate SGNs in order to convey the spectral and temporal cues of speech. These devices contain an array of up to 22 platinum electrodes that are inserted into the scala tympani - a fluid-filled canal within the cochlea - to electrically stimulate discrete populations of SGNs (Fig. 1). Cochlear implants take advantage of the tonotopic organisation of the SGNs; stimulation of electrodes in the apex of the cochlea evokes low pitch sensations while stimulation of electrodes positioned more basally evoke higher pitches. Implanted in over 300,000 patients around the world, cochlear implants are one of the most successful bionic devices developed; they are effective across the broad range of aetiologies associated with deafness and can be used in both adults and children.

While cochlear implant recipients typically receive significant benefit in speech understanding in quiet, performance decreases in noisy environments. In addition, these devices do not convey the rich aural texture of music or tonal languages very well due to the limited number of *independent* stimulation channels available as a result of current spread in the highly conductive fluid-filled cochlea that produces broad neural activation of SGNs. There are a number of approaches designed to improve the selectivity of electrical stimulation within the cochlea, including the use of current focussing techniques to create a more focused electrical field and the use of drug-based therapies to improve the electrode-neural interface via the application of exogenous neurotrophins to promote SGN neurite growth towards the electrode array (*4*).

In this issue of *Science Translational Medicine* Pinyon *et al*., describe a highly novel technique to deliver neurotrophins into the deaf cochlea using electroporation gene therapy

to transduce cells lining the scala tympani with a complementary DNA (cDNA) gene construct designed to drive the expression of brain-derived neurotrophic factor (BDNF). Electroporation is a means of transferring large molecules, such as cDNA, into cells by applying a series of brief but intense electric pulses to increase the permeability of the cell membrane. Significantly, these authors used the cochlear implant electrode array (Fig. 1) to generate the localized high electric field necessary to achieve the electroporation-mediated gene delivery. From a translational perspective this work is exciting as the authors have demonstrated both neurite growth into the scala tympani in the proximity of the electrode array and improved functional efficacy in the form of reduced electrical thresholds, using a technique that is transferrable to the clinic.

RESCUE OF AUDITORY NEURONS

Hair cells are sensitive to many forms of pathology and in mammals they do not regenerate; loss of hair cells is permanent. Importantly, SGNs undergo a sequence of pathological changes in response to hair cell loss, including the retraction of spiral ganglion neurites from the organ of Corti (Fig. 1) and eventually the degeneration of SGNs. The atrophic and degenerative changes to SGNs reflect the loss of endogenous neurotrophic factors, including BDNF, that are normally expressed by hair cells and support cells of the organ of Corti (*5*). As we have already noted, SGNs are the target neurons for cochlear implants, therefore the degeneration of their peripheral neurites, or loss of the neuron itself, reduces the efficacy of the electrode-neural interface as the neuron retracts from the electrode array. Over the past decade researchers have been exploring safe and effective ways of delivering exogenous neurotrophins to the deafened cochlea with the specific objective of improving

the electrode-neural interface. The study by Pinyon *et al*., is an important and novel contribution to this work.

While the inner ear has been a target for drug based therapies for over 60 years via systemic or middle ear routes, direct application to the cochlea has only been recently used clinically and is restricted to patients with a moderate–severe hearing loss where the risk of further damage to their hearing is small. These delivery techniques are usually designed to be performed in association with cochlear implantation as the scala tympani is already surgically exposed for the implantation of the electrode array. Techniques evaluated to date in preclinical studies include the drug eluting from the electrode array carrier; drug release from a reservoir and cannula within the electrode array; viral mediated gene therapy techniques; cell-based therapies; and nanotechnology inspired release techniques either as a polymer coating on the electrode array or via slow release particles that are inserted into the cochlea just before or after the insertion of the electrode array (*6-8*). While a number of these techniques have demonstrated SGN rescue and evidence of a functionally improved electrode-neural interface, they also bring potential clinical complications such as the risk of infection associated with drug reservoir and cannula systems, and the potential for off-target effects associated with the uncontrolled spread of viral mediated DNA delivery.

NEURON RESCUE USING ELECTROPORATION

This new work describes a technique to deliver a naked cDNA construct driving expression of the neurotrophin BDNF in mesenchymal cells that line the wall of the scala tympani. A novel aspect of this work is the use of the cochlear implant electrode array to deliver the "closed field" electroporation stimulus. The authors initially used *ex vivo* techniques to

evaluate the feasibility of the approach and to optimise the stimulus parameters required for efficient electroporation and confirmed that the technique optimally targeted mesenchymal cells lining the scala tympani proximal to the electrode array. Once proof of concept was demonstrated the approach was evaluated *in vivo* using normal hearing guinea pigs to again demonstrate the selective transduction of mesenchymal cells. Finally, the experiment was repeated in deafened animals that were chronically implanted with an electrode array following electroporation. The electrode array was activated two weeks following gene delivery and the electrically-evoked auditory brainstem response was recorded to monitor the functional status of the SGNs. Importantly, the mean threshold of the BDNF treatment group was significantly lower than the control cohort thereby demonstrating an important functional advantage that has also been reported using pump-based delivery techniques (*9-11*). The observed functional advantage was consistent with the anatomical findings showing a significant increase in both SGN soma area and neurite outgrowth towards the electrode array in the BDNF group after a two week treatment period. Taken together, these data show that the technique can result in an improved electrode-neural interface in deafened cochlea. It is anticipated that an improved interface will contribute to improved selectivity of electrical stimulation associated with cochlear implants.

A number of important questions still remain. First is the long-term efficacy of the approach. Pinyon *et al*., demonstrated neurite regrowth and functional advantage over the first two weeks following the treatment but described a reduction in BDNF expression over the three to six weeks post-gene delivery period. A key question associated with efficacy of the technique is the duration of any functional advantage after loss of the neurotrophin

expression. To this end, there is evidence that chronic depolarization of SGNs potentiates the rescue effects of exogenous neurotrophins (*11*); therefore the long-term efficacy of this technique needs to be evaluated in concert with chronic electrical stimulation via a cochlear implant. In addition, the longevity of the mesenchymal cells that take up the plasmid is unknown as is their status following a long-term hearing loss. Techniques designed to improve the longevity of BDNF expression and methods that allow targeting of other cells within the cochlea also need to be explored. In this new work the authors describe ectopic sprouting of SGN neurites in response to the BDNF treatment. This is a common finding associated with exogenous delivery of neurotrophins that has raised concerns about the maintenance of the cochlea's tonotopic organisation. Although evidence to date indicate that this organisation is not adversely affected (*12*), additional long-term experiments are warranted. Finally, the optimal stimulus parameters for effective electroporation need to be explored within the constraints of the specifications of the cochlear implant stimulator.

These questions aside, this new work presents a highly novel gene delivery technique. Importantly, the method is readily translatable to the clinic and requires only a minor modification in surgical procedure. One very exciting outcome is the potential to apply the technique to other medical bionics applications such as retinal prostheses and deep brain stimulation. Like cochlear implants, these devices electrically stimulate damaged nerves; the safe and local application of therapeutic drugs delivered via electroporation has the potential to improve the clinical outcomes for patients using a wide variety of prosthetic devices.

Figure 1. Schematic showing a normal cochlea (A) with an intact organ of Corti (OoC) containing the sensory hair cells (blue); spiral ganglion neurites that synapse onto the hair cells (neurites); and the spiral ganglion neuron soma (SGN). The organ of Corti degenerates in a sensorineural hearing loss (B), inducing the neurites to retract and some SGNs to degenerate. Patients with a widespread loss of hair cells receive no benefit from amplification via a hearing aid; the only therapy available to them is a cochlear implant where an electrode array is inserted into the scala tympani to directly stimulate SGNs (C). Pinyon *et al*., injected a DNA construct that expressed the neurotrophin BDNF into the cochlea which was taken up by mesenchymal cells lining the scala tympani (green) using electroporation via the cochlear implant electrode array. Ectopic neurite projections towards the electrode array were observed in concert with a reduction in electrical thresholds.

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