

Recent advancements toward gapless neural-electrode interface post-cochlear implantation

Crystal Y. Li, Rahul Mittal, Jenna Bergman, Jeenu Mittal, Adrien A. Eshraghi*

Cochlear implants (CI) are widely used to provide auditory rehabilitation to individuals with moderate to severe sensorineural hearing loss (Eshraghi et al., 2012). The scala tympani (ST) of the cochlea is the site of implantation of the intracochlear electrode array. In a healthy, normal ear, the cell bodies of the spiral ganglion neurons (SGNs) reside in Rosenthal's canal, a small cavity adjacent to the ST. SGNs have a peripheral neurite that projects to the hair cells on the basilar membrane of the organ of Corti, and a central axon that projects to the brainstem via the auditory nerve (Landry et al., 2013). From SGN cell bodies, the dendrites extend through the modiolus and the osseous spiral lamina to make synaptic contact with hair cells in the organ of Corti (Rusznák et al., 2009). In severe to profound deafness, the cochlea has few to no hair cells (Shibata et al., 2010). A CI helps overcome the problem of functional hair cells by directly stimulating the SGNs in the inner ear via short biphasic electric pulses (Li et al., 2017).

In the CI field, there is an increased interest in how preserving and restoring the functionality and number of SGNs may contribute to the success of CI for providing auditory rehabilitation (Shibata et al., 2010; Li et al., 2017). An anatomical gap between the electrode array and the auditory neurons in the inner ear impedes optimal electrical stimulation with CI. Hence, current devices are limited by 1) inadequate spatial specificity of inputs, thus suboptimal sound quality; and 2) large stimulation currents, thus high energy consumption (Wilson and Dorman 2008; Senn et al., 2017). Overlapping electrical fields, interference between channels, and spread of excitation from the electrodes lead to low resolution and low specificity of neuronal stimulation. This may be one of the major reasons for suboptimal sound quality as well as variability of speech and music perception. The gap between the electrode array and the auditory neurons leaves insufficient bridging between the perilymph-surrounded electrode contacts in the scala tympani and the SGNs in Rosenthal's canal of the bony modiolus. The CI must activate neurons located some distance from the electrode. To cross the gap and reach the SGNs, greater stimulation currents are needed. This structural gap also limits the count of possible non-overlapping stimulation points. SGNs receive inputs from a broad spectrum of frequencies, resulting in poorer frequency discrimination of sounds such as speech and music. Physical contact could potentially minimize current spread and enable the use of smaller currents to reach the stimulation thresholds of the contiguous auditory neurons (Li et al., 2017). In this perspective, we discuss the recent advancements toward gapless neural-electrode interface (Figure 1) post-CI.

Neurotrophins (NTs) are a class of growth factors that induce the survival, development, and function of neurons. Cochlear hair cells are

the primary source of endogenous NT peptides. A deficit of neurotrophic factors following the loss of hair cells in the deafened cochlea markedly reduces the number of peripheral fibers and SGNs via apoptosis. One such factor is pleiotrophin (PTN), a NT for different types of neurons expressed in the postnatal mouse cochlea. PTN knockout mice exhibit severe deficits in auditory brainstem responses, which signifies the importance of PTN in inner ear development and function, thus making it a promising candidate to support the viability of SGNs (Bertram et al., 2019). Both spiral ganglion cell explants and dissociated SGNs were cultivated with PTN *in vitro* at varying dilutions of 1:4, 1:8, and 1:16. While PTN showed a beneficial effect on neurite length and number of dissociated SGNs at dilutions of 1:4 and 1:8, no statistically significant effect was found for SGN neurites in organotypic explants (Bertram et al., 2019).

Brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are also key NTs expressed in the normal cochlea and have been studied as possible treatments to rescue SGNs from degradation (Shibata et al., 2010; Landry et al., 2013). Exogenous BDNF and NT-3 delivery over 27 days in an *in vivo*, deafened guinea pig model was shown to produce significant peripheral neurite growth (taken as measurements of length, lateral deviation, and directionality) compared to the deafened control groups that not receive NTs (Landry et al., 2013). Treatment with both NTs and electrical stimulation (ES) significantly increased the length in newly-sprouted neurites, compared to the unstimulated groups. Starting from post-implant day 4, ES was given continuously over 28 days, delivered sequentially as charge-balanced biphasic current pulses (1200 pulses per second per channel, stimulus intensity between -3 to +6 decibel (DB) per channel) (Landry et al., 2013).

Of clinical relevance, new neurite proliferation showed significantly lowered excitation thresholds in NT-treated animals (Landry et al., 2013). A lower stimulation threshold would require less power per pulse and thus reduce CI battery consumption. Interestingly, while Landry et al. (2013) did not show significantly increased spread of excitation as a result of the ectopic neurite growth, further study is warranted. Higher NT concentrations and/or longer treatment periods may induce disorganized neurite growth, potentially altering spatial excitation patterns and impacting perception of the acoustic environment.

With the goal of a closer interface between the electrodes and the neural population in mind, improvements in design and materials of guided regeneration of SGNs are also an active area of study. Regenerating auditory neurons must traverse the perilymph barrier to reach the CI electrode. Biodegradable calcium phosphate hollow nanospheres display promise as a potential avenue for sustained, long-term

release of growth-promoting NTs when coated onto CI electrodes (Li et al., 2017). Using a 3D *in vitro* culture model, it was shown that the regenerating auditory neuron dendrites were attracted by targeted NT release and were able to establish direct physical contact between the auditory neurons and the CI electrodes (Figure 1). The calcium phosphate hollow nanospheres were coated onto CI electrodes and loaded with NTs. Calcium phosphate hollow nanosphere capacity for uptake and release of NTs was determined using ¹²⁵I-conjugated glia cell line-derived neurotrophic factor. Neurites from human vestibulocochlear ganglion explants reached and established physical contact with the glia cell line-derived neurotrophic factor-loaded calcium phosphate hollow nanospheres coating on the CI electrodes positioned 0.7 mm away. 3D-reconstruction of the Z-stacked images showed that Tuj1 (a nerve development marker) positive neuronal extensions took root on the coating and grew to reach the electrodes (Li et al., 2017). These axon guidance effects suggest NT delivery via calcium phosphate hollow nanospheres coating may be a key toward a gapless neural-electrode interface.

In addition to targeted sustained release, long-term NT administration is necessary for protective benefits to auditory neurons. Delivery via NT injection is generally short term, and repeated injections are not ideal due to risk of infection. However, implantation of mesenchymal stem cells (MSCs) genetically modified to overexpress BDNF may be a feasible drug delivery system. The stem cells encapsulated in alginate protect against host immune response and prevent their unrestrained migration (Schwieger et al., 2020). A physiologically stable hydrogel that allows bidirectional transfer of small molecules in (nutrients, growth factors, oxygen), and out (waste products, insulin, BDNF) can further facilitate continuous NT release in the setting of neurite preservation and regrowth. Alginate, a polysaccharide isolated from bacteria or the cell wall of brown algae, is one such material which meets the above-mentioned criteria (Schwieger et al., 2020). Ultrahigh viscous alginate is even more suited for medical applications as it fulfills the requirements of high molecular weight, low endotoxin levels, and sterility. There is no alginate-degrading enzyme in humans. Using an *in vitro* dissociated rat SGN co-culture model, alginate-mesenchymal stem cell samples were electrically stimulated, and alginate stability as well as MSC survival were monitored. Electrical stimulation of "biphasic 800 μ s pulses (400 μ s per phase) and 120 μ s interpulse gap for 24 hours in an incubator" (Schwieger et al., 2020) was used. After 21 days, 330 μ A of this electrical stimulation did not affect the viability or survival of MSCs within the investigated time frame compared to unstimulated controls (Schwieger et al., 2020). However, it was not mentioned whether the electrical stimulation was charge balanced or not. Alginate stability was also tested using red fluorescence from the tdTomato marker protein; reduction in fluorescence indicative of damage of the stimulated alginate-embedded cells was not seen.

Does long-term NT delivery (via elution from mesenchymal stem cells (MSCs) compared to short-term NT delivery (via injection) produce a tangible difference in SGN survival? In an *in vivo* model of systemically deafened guinea pigs, two such application strategies were evaluated. BDNF-overexpressing MSCs were

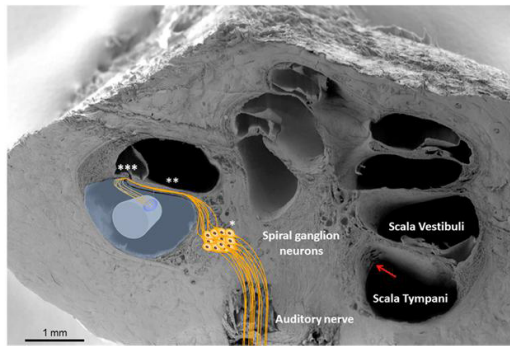


Figure 1 | An illustration of spiral ganglion neurons guided toward a cochlear implant electrode.

By attracting neurons using neurotrophic stimulation and replacing the perilymph with an extracellular gel matrix, the anatomical gap between the auditory nerve and electrode could be closed. Peripheral dendrites grow from modiolus (*) via osseous spiral lamina (**) and through habenula perforata (***) to scala tympani. Another route is growing directly through canaliculi perforantes (arrow). Reduced distance will result in minimized current spread from the cochlear implant, enabling the use of a higher number of non-overlapping stimulation points (adapted from Rask-Andersen et al., 2012; reprinted from Li et al., 2017 with permission from Elsevier).

encapsulated in an “ultrahigh viscous alginate matrix” and were either “injected into the scala tympani or used to coat the cochlear implant array” (Scheper et al., 2020). Neither electrode impedance, fibrosis, nor average hearing threshold were affected by the alginate-MSc injection or the MSc coating compared to normal-hearing controls. MScs were able to survive the 28-day implantation period. Four weeks after implantation into deafened ears, the SGN density and survival rate were found to be significantly higher in the alginate-MSc-coated CI group (16.30 ± 0.64 SGN/10,000 μm^2) than the deafened controls (10.91 ± 0.52 SGN/10,000 μm^2 , $P < 0.05$). In comparison, injection of alginate-MScs did not show a SGN survival benefit (11.61 ± 1.54 SGN/10,000 μm^2). In fact, the injection group resulted in significantly lower SGN survival compared to the alginate-MSc-coated CI group (Scheper et al., 2020). BDNF-producing MScs enveloped in ultrahigh viscous alginate prevented SGN degradation in the form of coating on the CI surface, but not in the form of an injection (Scheper et al., 2020).

Other media have also shown promise as a matrix to promote neurite sprouting. Decellularized equine carotid artery tissue has been explored as one such medium (Yilmaz-Bayraktar et al., 2020). Arteries are composed of three tissue layers: (1) tunica adventitia, the thick outermost layer, which is made of loose connective tissue and fibroblasts; (2) tunica media, the middle layer, which consists mainly of circumferentially arranged smooth muscle cells; (3) the innermost tunica intima, which is comprised of a layer of endothelium lining the vessel lumen supported by a subendothelial layer of loose connective tissue. In a study by Yilmaz-Bayraktar et al. (2020), rat SGN explants were cultured on decellularized equine carotid artery layers and neurite sprouting was assessed quantitatively. Neurite outgrowth was most notable on the intima, followed by the adventitia and the least growth on the media. Unlike a more randomized structure of the tunica adventitia, the intima displays the smooth surface of the basal membrane, which in particular seems to facilitate the adhesion and the outgrowth. Additionally, the intima’s low immunogenicity and high biocompatibility make it suitable for supporting neurite growth (Jeinsen et al., 2018). Increased neurite outgrowths on the tunica intima may be also due to the presence of laminin in the matrix and the support of endothelial $\beta 1$ integrins

which resisted the detergent-based process of decellularization (Yilmaz-Bayraktar et al., 2020).

In summary, recent findings suggest that NT-3, BDNF, glia cell line-derived neurotrophic factor and PTN are promising endogenous NTs for protection and sprouting of auditory neuron dendrites. However, further studies are required to determine the long-term safety of these NTs and any potential side effects. It is expected that a NT combination will be more efficient in inducing neurite sprouting compared to an individual NT alone. The *in vivo* studies discussed here had experimentation periods of 1 month or less, so long-term studies are necessary. Of particular interest is *in vivo* survival duration of MScs in the alginate coating, whether the protective effect on neural elements can be sustained for longer periods, and the effect of higher NT concentrations with longer treatments. Repurposing pharmaceutical compounds already used in humans which can induce the production of these NTs as well as promote neurite sprouting holds a great potential in developing strategies for gapless neural-electrode interface. In addition, these pharmaceutical compounds can be coated onto the electrodes or incorporated inside the electrodes (drug eluting electrode), thus addressing the potential challenges associated with the delivery of NTs.

The preclinical animal models continue to be necessary and of immense value in areas of investigation surrounding biochemical and biophysical mechanisms to create a gapless neural-electrode interface. The potential future direction of research using these preclinical models would be to determine whether regrown neurites will be functionally relevant. Developing strategies to create a gapless neural-electrode interface will improve the quality of life of the individuals who receive cochlear implantation and their family members.

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Crystal Y. Li, Rahul Mittal, Jenna Bergman, Jeenu Mittal, Adrien A. Eshraghi*

Department of Otolaryngology, University of Miami Miller School of Medicine, Miami, FL, USA (Li CY, Mittal R, Bergman J, Mittal J, Eshraghi AA) Department of Neurological Surgery, University of

Miami Miller School of Medicine, Miami, FL, USA (Eshraghi AA)
Department of Biomedical Engineering, University of Miami, Coral Gables, FL, USA (Eshraghi AA)
***Correspondence to:** Adrien A. Eshraghi, MD, MSc, FACS, aeshraghi@med.miami.edu.
<https://orcid.org/0000-0002-1559-8573> (Adrien A. Eshraghi)
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References

- Bertram S, Roll L, Reinhard J, Groß K, Dazert S, Faissner A, Volkenstein S (2019) Pleiotrophin increases neurite length and number of spiral ganglion neurons in vitro. *Exp Brain Res* 237:2983-2993.
- Eshraghi AA, Nazarian R, Telisch FF, Rajguru SM, Truy E, Gupta C (2012) The cochlear implant: historical aspects and future prospects. *Anat Rec (Hoboken)* 295:1967-1980.
- Jeinsen N, Mägel L, Jonigk D, Klingenberg M, Haverich A, Wilhelm M, Böer U (2018) Biocompatibility of intensified decellularized equine carotid arteries in a rat subcutaneous implantation model and in a human in vitro model. *Tissue Eng Part A* 24:310-321.
- Landry TG, Fallon JB, Wise AK, Shepherd RK (2013) Chronic neurotrophin delivery promotes ectopic neurite growth from the spiral ganglion of deafened cochleae without compromising the spatial selectivity of cochlear implants. *J Comp Neurol* 521:2818-2832.
- Li H, Edin F, Hayashi H, Gudjonsson O, Danckwardt-Lillieström N, Engqvist H, Rask-Andersen H, Xia W (2017) Guided growth of auditory neurons: Bioactive particles towards gapless neural-electrode interface. *Biomaterials* 122:1-9.
- Rask-Andersen H, Liu W, Erixon E, Kinnefors A, Pfäller K, Schrott-Fischer A, Glueckert R (2012) Human cochlea: anatomical characteristics and their relevance for cochlear implantation. *Anat Rec (Hoboken)* 295:1791-1811.
- Rusznák Z, Szucs G (2009) Spiral ganglion neurons: an overview of morphology, firing behaviour, ionic channels and function. *Pflügers Arch* 457:1303-1325.
- Scheper V, Hoffmann A, Gepp MM, Schulz A, Hamm A, Pannier C, Hubka P, Lenarz T, Schwieger J (2019) Stem cell based drug delivery for protection of auditory neurons in a guinea pig model of cochlear implantation. *Front Cell Neurosci* 13:177.
- Schwieger J, Hamm A, Gepp MM, Schulz A, Hoffmann A, Lenarz T, Scheper V (2020) Alginate-encapsulated brain-derived neurotrophic factor-overexpressing mesenchymal stem cells are a promising drug delivery system for protection of auditory neurons. *J Tissue Eng* 11:2041731420911313.
- Senn P, Roccio M, Hahnwald S, Frick C, Kwiatkowska M, Ishikawa M, Bako P, Li H, Edin F, Liu W, Rask-Andersen H, Pyykkö I, Zou J, Mannerström M, Kepner H, Homsy A, Laux E, Llera M, Lellouche JP, Ostrovsky S, et al. (2017) NANOCI-nanotechnology based cochlear implant with gapless interface to auditory neurons. *Otol Neurotol* 38:e224-231.
- Shibata SB, Cortez SR, Beyer LA, Wiler JA, Di Polo A, Pfingst BE, Raphael Y (2010) Transgenic BDNF induces nerve fiber regrowth into the auditory epithelium in deaf cochleae. *Exp Neurol* 223:464-472.
- Yilmaz-Bayraktar S, Schwieger J, Scheper V, Lenarz T, Böer U, Kreienmeyer M, Torrente M, Doll T (2020) Decellularized equine carotid artery layers as matrix for regenerated neurites of spiral ganglion neurons. *Int J Artif Organs* 43:332-342.

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