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Guided growth of auditory neurons: Bioactive particles towards gapless neural – electrode interface

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ABSTRACT

Cochlear implant (CI) is a successful device to restore hearing. Despite continuous development, frequency discrimination is poor in CI users due to an anatomical gap between the auditory neurons and CI electrode causing current spread and unspecific neural stimulation. One strategy to close this anatomical gap is guiding the growth of neuron dendrites closer to CI electrodes through targeted slow release of neurotrophins. Biodegradable calcium phosphate hollow nanospheres (CPHSs) were produced and their capacity for uptake and release of neurotrophins investigated using ^{125}I-conjugated glia cell line-derived neurotrophic factor (GDNF). The CPHSs were coated onto CI electrodes and loaded with neurotrophins. Axon guidance effect of slow-released neurotrophins from the CPHSs was studied in an *in vitro* 3D culture model. CPHS coating bound and released GDNF with an association rate constant 6.3×10^3 $M^{-1}s^{-1}$ and dissociation rate 2.6×10^{-5} s^{-1}, respectively. Neurites from human vestibulocochlear ganglion explants found and established physical contact with the GDNF-loaded CPHS coating on the CI electrodes placed 0.7 mm away. Our results suggest that neurotrophin delivery through CPHS coating is a plausible way to close the anatomical gap between auditory neurons and electrodes. By overcoming this gap, selective neural activation and the fine hearing for CI users become possible.

1. Introduction

Cochlear implants (CIs) have been used to restore hearing in patients with profound hearing loss for several decades. The results are generally good, especially in children. A CI bypasses a dysfunctional sensory epithelium and directly stimulates the spiral ganglion neurons with short biphasic electric pulses. The first multi-electrode CIs were developed to treat patients with profound sensorineural hearing loss in the 1970's [1]. Thereafter, there have been continuous updates with different coding strategies to improve the conversion of sound into digital signals that can be interpreted by the auditory nerve [2]. The electronics (processor and receiver) have also been improved whilst the design of the electrode arrays have undergone rather few modifications. The functional outcome with CIs varies between patients and seems to have reached a plateau; no improvement, as to mean recognition of monosyllabic words in post-lingual deafened adults, has been achieved for decades [3,4].

One problem is the inability of CIs to deliver independent signals to individual spiral ganglion neurons [5,6]. Efforts were made to organize the growth of neurites on implant surfaces and increase the total electrode count [7,8], as CIs typically employ 12–22 electrodes to replace the function of 3400 auditory inner hair cells. Although promising, this strategy requires the auditory neurons to have direct contact with the implant electrode. A first obstacle is the anatomical gap between the CI electrode and the auditory neurons; the electrode is inserted into the perilymph-filled scala tympani, through which neurons cannot grow. Edin et al. (2014)

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demonstrated that re-sprouted human vestibulocochlear neurons can grow through gels, which opened up possibilities for regenerating auditory neurons to traverse the perilymph barrier and reach the CI electrode [9]. Perilymph would thus be replaced with a nerve-stimulating gel to bridge the gap between the auditory neurons and the CI electrode. An even harder task remains to guide the growth of auditory neurons through the gel and form direct physical contact with the CI electrode. If such an interface was accomplished, it could result in lower stimulation thresholds, which in turn allows for selective neural stimulation and improved sound quality and speech perception. A lower stimulation threshold would also necessitate less power in each pulse and would reduce CI battery consumption – another bottleneck of the device [5,10–12].

As the CI depends on functional spiral ganglion neurons, extensive research on their survival, regeneration, and guidance has been conducted, including attracting and repelling guidance cues. Netrin-1 attracted growth cones from regenerating guinea pig spiral ganglion neurons *in vitro* [13]. Brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) increased the innervation of separated murine organ of Corti explants when co-cultured with spiral ganglion neurons [14]. Several *in vivo* studies have confirmed these results by successfully regenerating peripheral extensions in animals with ablated hair cells [15–18]. Regeneration based on long-term chronic infusion of nerve growth factors [16] may not be desirable in clinic because of the risk of infections. Additional strategies involve adenoviral gene therapy to overexpress glia cell line-derived neurotrophic factor (GDNF) and protect hair cells [19]. Close-field electroporation of gene fragments for producing BDNF using a CI resulted in regeneration of spiral ganglion neurites in a guinea pig model. Neurites extended closer to the CI electrode and yielded lower stimulus thresholds in a CI array [17]. Another strategy to provide long-term administration is to coat a CI with cells genetically modified to overexpress neurotrophic compounds [20]. However, these approaches do not necessarily provide the desired gradient stimulating physical contact between spiral ganglion neurons and CI, since neurotrophins are randomly expressed. Safety issues also remain to be solved before considering clinical applications. Therefore, a combination of targeted neurotrophin release and neural growth stimulating gel could be a more reasonable strategy, as the delivery of neurotrophins using mesoporous silica supraparticles has already been shown to prevent the death of spiral ganglion neurons in the guinea pig model [18].

Here, we developed a delivery approach to coat CI electrodes with calcium phosphate hollow nanospheres (CPHSs) loaded with neurotrophins. Calcium phosphates have been used as drug carriers and hollow spheres can host drugs with high loading and sustained release. Calcium phosphates were proven to be suitable for the delivery of high-molecular-weight growth factors [21,22]. From safety aspects, calcium phosphates are the main components in human bones and teeth, making them ideal candidates for clinical use. Previous studies show CPHSs are synthesized via a mineralization process under high temperatures [23]. It has been used in ranges of bone grafts in healing models with no signs of toxicity [24,25]. To date, calcium phosphate is clinically used, e.g. as bone fillers. It is not known whether calcium phosphate has been used in the cochlea, but hydroxyapatite is frequently used to repair the vestibular part of the temporal bone treating patients with superior semicircular canal dehiscence without showing adverse effects [26]. Calcium phosphate is also used in dietary supplements and in approved drugs such as Calfovit D3. It can be degraded *in vivo* and is considered to be safe when calcium and phosphate ions are released in the human body.

By coating CI electrodes with neurotrophin-loaded CPHSs, we believe a sustained release of the neurotrophins can be established when operated into patients. These guidance cues would be able to attract the growth of regenerating auditory neuron dendrites through bioactive gels and finally establish direct physical contact between the auditory neurons and the CI electrodes (Fig. 1). To verify our hypothesis, an *in vitro* sustained release 3D model was

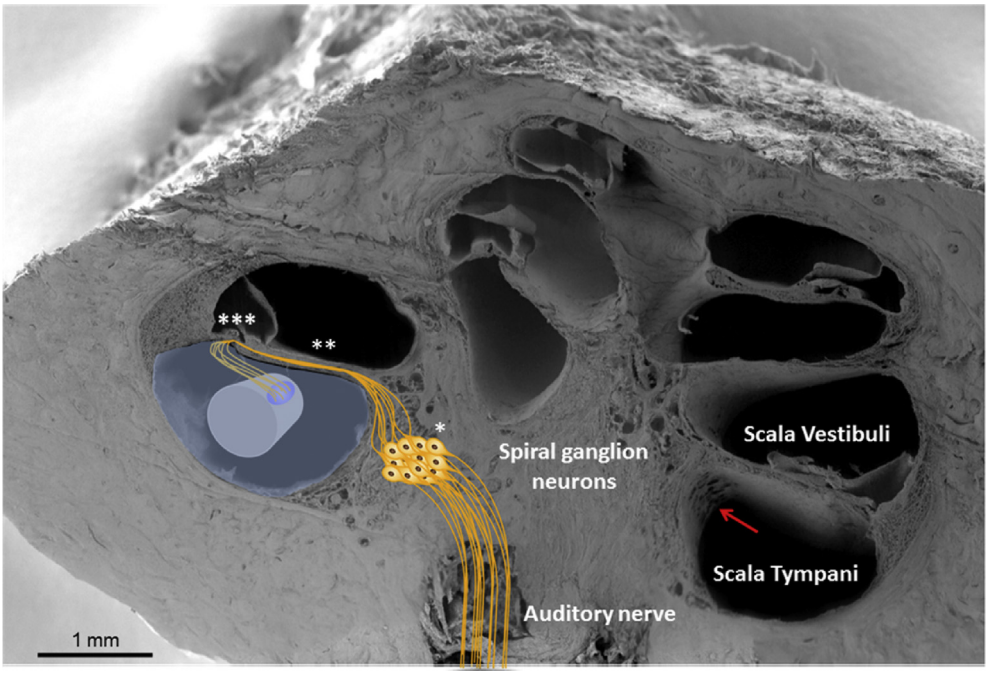

Fig. 1. An illustration of spiral ganglion neurons guided toward a CI electrode. By attracting neurons using neurotrophic stimulation and replacing the perilymph with an extracellular gel matrix, the anatomical gap between the auditory nerve and electrode could be closed. Peripheral dendrites grow from 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 CI, enabling the use of a higher number of non-overlapping stimulation points (adapted from Rask-Andersen et al., 2012).