Emerging therapies for human hearing loss

Abstract

Introduction: More than 5% of the world's population have a disabling hearing loss which can be managed by hearing aids or implanted electrical devices. However, outcomes are highly variable and the sound perceived by recipients is far from perfect. Sparked by the discovery of progenitor cells in the cochlea and rapid progress in drug delivery to the cochlea, biological and pharmaceutical therapies are currently in development to improve the function of the cochlear implant or eliminate the need for it altogether.

Areas Covered: This review highlights progress in emerging regenerative strategies to restore hearing and adjunct therapies to augment the cochlear implant. Novel approaches include the reprogramming of progenitor cells to restore the sensory hair cell population in the cochlea, gene therapy and gene editing to treat hereditary and acquired hearing loss. A detailed review of optogenetics is also presented as a technique that could enable optical stimulation of the spiral ganglion neurons, replacing or complementing electrical stimulation.

Expert Opinion: Increasing evidence of substantial reversal of hearing loss in animal models, alongside rapid advances in delivery strategies to the cochlea and learnings from clinical trials will amalgamate into a biological or pharmaceutical therapy to replace or complement the cochlear implant.

Key Words

Cell-based Therapy, Cochlear Implant, Hair Cell, Hearing Loss, Gene Therapy, Optogenetics, Regeneration, Spiral Ganglion Neuron

Article Highlights

- There is currently no approved pharmaceutical or biological therapy to reverse hearing loss
- A number of preclinical studies have shown improved auditory function through the preservation or regeneration of cochlear sensory cells by various strategies based on the pathology of hearing loss
- Genetic and pharmacologic manipulation of the Notch and Wnt signalling pathways result in regeneration of cochlear hair cells
- Application of neurotrophic factors to the cochlea can repair the hair cell ribbon synapse that is damaged by noise over-exposure
- Effective reversal of hearing loss has been demonstrated via timely application of gene therapy to introduce normal copies of genes into cells or gene editing techniques for targeted gene disruption or repair of mutations
- Strategies to improve cochlear implant function include improving the nerve-electrode interface and using optogenetics to make neurons responsive to light to allow the use of optical cochlear implants

1.0 Hearing loss

Hearing loss affects a staggering 1.5 billion people worldwide, and is projected to affect 2.5 billion people by 2050 [1]. It can have a significant impact on an individual's education and employment. Difficulties in communication with others can reduce self-esteem and confidence and cause social withdrawal which may lead to mental health issues [1].

The peripheral hearing pathway begins at the outer ear where the pinna picks up sound waves and funnels them through the ear canal to vibrate the eardrum. The motion of the eardrum is mechanically transmitted through the middle ear via the ossicles, with the smallest of these bones, the stapes, connected to the cochlea of the inner ear via the oval window membrane. The movement of the oval window translates to vibration of the basilar membrane. This motion stimulates the cochlear hair cells, generating nerve impulses in spiral ganglion neurons which transmit to the auditory cortex via the brainstem. The sensorineural and structural elements of the cochlea and the ascending auditory pathway are shown in **Figure 1**.

There are four forms of hearing loss (sensorineural, conductive, combined, and central), but the majority can be classified as sensorineural, with an underlying pathology of the hair cells and/or the spiral ganglion neurons. Sensorineural hearing loss is classified as mild, moderate, severe, profound, or total, and can be caused by any of several pathophysiological mechanisms. The most common cause of hearing loss in adults is age-related hearing loss [2], in which there is gradual degeneration of sensory cells or neural pathways over time. Other factors causing hearing loss include genetics, noise exposure, infection, adverse perinatal conditions (e.g. hypoxia, asphyxia and ischemia), trauma, medications and toxins, and dysfunction of spiral ganglion neurons [1]. Other forms of hearing loss are based on conductive dysfunction, central auditory processing disorders or a combination of these [1].

Cochlear hair cells do not spontaneously regenerate in mammals, meaning that any trauma to hair cells can result in permanent hearing impairment [3]. Permanent sensorineural hearing loss may be managed through the use of hearing aids, implanted devices (cochlear implant, bone conduction implant, middle ear implant, auditory brainstem implant), smartphone apps, sign language, closed captions, and lip reading [4], but none of these address the underlying cause of the hearing loss.

2.0 Current treatments for hearing loss

Management strategies for hearing loss depend upon the etiology and severity of hearing loss, as well as patients' personal needs. Hearing aids are commonly prescribed for patients with some remaining hearing and are the most commonly used non-invasive treatment. A hearing aid is a small electronic device fitted to the outer ear that detects sounds via a microphone, processes and amplifies it, then delivers the sound directly to the ear canal. In doing so, the audibility of sounds is improved, permitting users to better perceive the acoustic environment. In cases where the ear canal or middle ear is dysfunctional, or the hearing aid is not suitable for the user (e.g. due to lifestyle choices or recurring ear infection), an implanted device may be recommended.

Implanted devices that may be used in place of a hearing aid include active middle ear implants, or bone conduction implants. An active middle ear implant is a fully implantable device that bypasses the ear canal, attaching to and moving either one of the ossicles of the middle ear, or the oval window, to activate the cochlear hair cells. Alternatively, a bone conduction implant bypasses both the outer and middle ear, instead using vibrations of the skull to activate the hair cells of the cochlea. As with hearing aids, both active middle ear implant and bone conduction implant devices require the presence of some functional sensory hair cells to be effective.

In cases of severe, profound or total hearing loss, cochlear implants may be utilised to artificially restore hearing [5,6]. The cochlear implant (CI) is a medical device that consists of implanted electrodes that are used to electrically stimulate the spiral ganglion neurons of the cochlea, bypassing the damaged or missing sensory hair cells. The CI has an external component fitted behind the ear that has a microphone to detect environmental sounds and a speech processer to filter the sound into frequency bands. This information is transmitted through the skin via an external transmitting coil and an internal receiver/stimulator which are held in proximity magnetically. The signals are mapped to specific electrodes which are implanted into the scala tympani (**Figure 2A**). In normal hearing listeners, each part of the basilar membrane vibrates maximally to a characteristic frequency. To emulate this in the CI, high-frequency signals are transmitted to the electrodes at the basal end of the cochlea and low-frequency signals are sent to the electrodes at the apical end of the cochlea. While place of excitation is expected to provide some pitch information to a CI recipient, pitch is also encoded by timing properties of the neural response whereby the temporal firing pattern of spiral ganglion neurons is dependent on the temporal pattern of oscillations of the sound. CI stimulation strategies typically employ temporal amplitude modulations for each frequency band, varying the current level of a fixed-rate pulse train at the relevant electrodes.

Individuals with residual functional low frequency hearing but significant high frequency hearing loss may benefit from combined electric and acoustic stimulation (EAS). EAS uses electrical stimulation (i.e. a CI) for high frequencies and acoustic stimulation (natural or amplified) for low frequencies in the same ear simultaneously. This is different from bimodal hearing which uses a CI in one ear and acoustic hearing (with or without amplification) in the other. The input frequency at which the stimulator switches from acoustic amplification to electric stimulation is known as the crossover frequency. There may also be a region of overlap frequencies, which are assigned to both acoustic and electric stimulation. The width of the overlap, if any, is dependent on user preference and their individual hearing profiles [7].

For patients where a CI is not suitable, such as those where the cochlea and/or auditory nerve are completely absent or severely damaged, an auditory brainstem implant may provide a means for hearing restoration. An auditory brainstem implant is a fully implanted device which uses an external system and internal receiver like the CI. However, instead of using an electrode array in the cochlea, up to 21 auditory brainstem implant electrodes are placed on the surface of the cochlear nuclei (**Figure 1B**) of the brainstem to stimulate the auditory pathway.

2.1 Outcomes and limitations of current treatments

Clinical studies on the efficacy of bone conduction implants or active middle ear implants reveal these devices to be as effective as hearing aids, safe and generally resulting in good patient satisfaction [8-10]. Outcomes with auditory brainstem implants are more variable and dependent on etiology of hearing loss. Most patients achieve only sound awareness and environmental sound discrimination with a much smaller proportion of recipients achieving open set speech perception [11-15].

For the majority of adult post-lingual recipients (i.e., after development of oral communication) cochlear implantation significantly improves speech perception in quiet environments [16-18], and has a significant and positive effect on quality of life [19-22]. However, CIs cannot perfectly replicate normal hearing. Losses of spectral information (e.g., pitch) and temporal cues (e.g., rhythm) are experienced. Speech perception in noise is difficult for most people, but it is especially challenging for CI users [23-25]. Additionally, users often demonstrate difficulty identifying speaker gender [26,27], distinguishing questions and statements (i.e., sentence intonation) [28,29], recognising voice

emotion [30] and lexical tones [31-33]. Furthermore, most recipients report less enjoyment of music post implantation compared to before their loss of hearing or to normal hearing listeners [34-38].

Compared to a CI alone, EAS users demonstrate superior speech perception outcomes in both quiet and noisy environments [39]. Furthermore, studies report improved pitch discrimination and melody recognition with EAS over CI alone, although these studies do not exclude the contralateral partially hearing ear which may lead to overestimation of the benefit of EAS [40-42]. These results further emphasise the value of preserving residual hearing and spiral ganglion neuron health during cochlear implantation by reducing the inflammatory response (see Section 3.2.3), development of atraumatic surgical techniques and CI design.

A major contributing factor to the limitations of hearing with a CI is that the fluid of the cochlea is highly conductive. Current from the electrodes spreads broadly, overlapping with the stimulation area of adjacent electrodes, resulting in undesirable interactions, and therefore reduced independent spectral channels (**Figure 2B**). Efforts to reduce current spread through methods such as current shaping have to date proven ineffective in a clinical setting [43,44], and as such there is a need for the development of novel therapies that replace or augment the functionality of CIs.

3.0 Emerging treatments for hearing loss

The long-held belief that hair cells do not regenerate upon damage was challenged in the late 1980s by two landmark studies that demonstrated the potential for some non-mammalian vertebrates to make new hair cells [45,46], raising the possibility that this could be achieved in humans as well. These findings instigated an uprising in the auditory neuroscience field focused on applying novel regenerative medicine strategies to restore or prevent hearing loss (**Figure 3**). Subsequent discoveries that the inner ear harbors populations of progenitor cells that may be manipulated to regenerate into hair cells or neurons upon damage have further accelerated the pace of this research [47-49]. So far, several strategies to treat hearing loss have been tested including cell-based therapies, pharmaceuticals, or gene therapy, with some treatments already in clinical trials. This section will focus on the status of the research and progress of the key current clinical trials. For an in-depth review of all clinical trials regarding drug treatments for hearing loss, the authors recommend the systematic review by Crowson et al. (2017) [50].

3.1 Cell-based therapies

Transplantation of *in vitro*-derived sensory cells from embryonic or induced pluripotent stem cells to replace damaged cells in the cochlea have been actively tested as a treatment option for hearing loss. Significant effort has been invested in testing differentiation protocols to generate high hair cell yields [51]. Although engraftment of stem cell-derived hair cells into the sensory epithelium has been observed, no improvements in hearing function have been reported to date [52]. This is likely due to the difficulty in recapitulating the complexity of the sensory epithelium, including challenges in delivering the cells to the correct location (outer vs inner hair cells) and promoting their proper innervation. As such, the field is shifting towards applying stem cell-derived hair cells derived via organoid technology or alternate methods for understanding differentiation mechanisms and/or screening for drugs/genes that promote regeneration. Conversely, transplantation of stem cell-derived auditory neural progenitors has gained traction as a feasible therapeutic option to replace lost spiral ganglion neurons, particularly for the treatment of auditory neuropathies. Effective differentiation of embryonic and induced pluripotent stem cells towards spiral ganglion neurons *in vitro* and their enhanced survival *in vivo* have been reported [53-57]. In one key study, integration of embryonic stem cell-derived neurons into the damaged cochlea accompanied by marginal

improvements in hearing function was reported [56]. Further improvements in functional outcomes may be achieved with further characterisation of the optimal stage of differentiation required for transplantation, surgical route of cell delivery and testing alternate differentiation protocols [58-60]. If successful, this treatment may not be limited to auditory neuropathies alone, but could extend to improving outcomes with a CI, which rely on a sufficient population of neurons for its functionality [61].

3.2 Pharmaceuticals

Pharmacological intervention, specifically the application of small molecules or drugs, is another potential approach to regenerate hair cells or neurons in the deaf inner ear. This treatment has been tested for sensorineural hearing loss caused by complex etiologies such as noise exposure, ageing or antibiotics. Dramatic improvements in our understanding of inner ear development with advancements in molecular and sequencing technologies have resulted in the discovery of multiple signalling pathways and genes critical for hair cell and neural regeneration that may be targeted for regenerative therapies.

3.2.1 Hair cell regeneration

The Notch and Wnt signalling pathways have become key targets for hair cell regeneration, given their role in development in regulating the propensity for inner ear progenitors to acquire a hair cell fate over a supporting cell fate [62,63]. Pre-clinical studies showed that treatment of noise-deafened mice with a small molecule Notch inhibitor drug LY411575 led to partial improvements in hearing function and the generation of some "new" hair cells [64]. These promising findings led to the first-in-human clinical trial launched by REGAIN and Audion Therapeutics (ClinicalTrials.gov Identifier: NCT05061758 and EudraCT Number: 2016-004544-10). The clinical trial is currently in Phase 2, with early indications of efficacy in word-recognition scores in noise). Another competitor in this arena includes a drug developed by Frequency Therapeutics, FX-322, targeting the Wnt pathway and epigenetic modifier histone deacetylase (HDAC; ClinicalTrials.gov Identifier: NCT04629664). This drug combination was developed based on findings showing that manipulation of the Wnt pathway combined with epigenetic targeting activates proliferation and differentiation of inner ear progenitors [65,66]. Phase 1 clinical data showed that the drug is safe and well-tolerated, with evidence that of the six patients treated with FX-322, four had significant improvements in their word-recognition scores in quiet and noise conditions, relative to their baseline score [67]. Phase 2 is currently underway.

As supporting cells play a key role in cochlear homeostasis, promoting their proliferation rather than transdifferentiation is an alternate approach. Drug therapies to activate supporting cell proliferation in the cochlea by targeting the cell cycle inhibitor genes (p27kip1 or Rb gene) or activating the Hippo-Yap signalling pathways are also gaining traction [68-71]. Of note, recent studies have shown that targeting the Hippo-Yap signalling pathway with a small molecule drug TRULI elicits robust proliferation of supporting cells in the neonatal organ of Corti, as measured by the incorporation of EdU in neonatal mouse cochlear and utricular cultures [68]. However, the effectiveness of this approach has yet to be demonstrated in the adult cochlea.

3.2.2 Neural/synaptic regeneration

Acoustic trauma, even at moderate levels, can lead to an excitotoxic injury in which there is damage to the hair cell ribbon synapse without loss of the hair cell, often referred to as synaptopathy or hidden hearing loss as it is not always detected in regular hearing test screens [72]. Neurotrophic factors, particularly neurotrophin-3, have been shown to repair the hair cell ribbon synapse and

improve auditory function following acoustic trauma [73-75]. However, the hearing function outcomes between animals were variable [73]. Recently, some small molecule neurotrophin analogues/Trk antibodies have also shown promise in promoting synaptic regeneration, but here again, marginal functional improvements post-treatment have hindered the progress of the therapy [76-79]. The variable hearing function outcomes may be attributed to inconsistency in drug entry into the cochlea across the round window membrane, which is the safest and least-invasive route for local application of therapeutics. As such, there is a significant need to develop technologies to improve the reliability of drug delivery across the round window membrane and thereby therapeutic outcomes. Nanoparticle-based approaches or conjugation to bisphosphonates (as a mode of anchoring the drug to the cochlear bone) are a couple of examples of promising solutions to improving round window membrane drug delivery [80-82]. Nevertheless, a clinical trial has recently launched testing intratympanic delivery of brain-derived neurotrophic factor for patients with difficulty hearing speech in noise (Otonomy; ClinicalTrials.gov Identifier: NCT04129775).

Pharmacological treatments are also under consideration as an adjunct to cochlear implantation. A number of factors may reduce the efficacy of the CI including degeneration of spiral ganglion neurons that occurs in some forms of hearing loss [83,84], and fibrous tissue or bone growth around the electrode arrays [85], both of which degrade the nerve-electrode interface. The introduction of neurotrophic factors into the cochlea, via a slow-release system or a drug-eluting coating of the cochlear implant itself, has been shown to enhance the overall survival of spiral ganglion neurons after hearing loss and to encourage the growth of neuronal processes [86-89], thus closing the gap between the nerve and the electrode and lowering thresholds of activation [90]. However, the impact of this therapy is transient [91], leading researchers to explore gene therapy to employ the cells of the cochlea to continually release neurotrophins (see Section 3.3.2).

3.2.3 Reducing the foreign body reaction to the CI

Reducing inflammation and fibrosis is another avenue to preserve hearing during cochlear implantation and improve the nerve-electrode interface which are especially important for CI strategies that combine electrical and acoustic stimulation (see Sections 2.0 and 2.1). In preclinical studies, applying corticosteroids such as dexamethasone at the time of cochlear implantation, either systemically, locally to the round window or via drug-eluting CIs, was shown to suppress the inflammatory response initiated by surgery, reduce the formation of fibrous tissue and bone growth, often also preventing loss of hearing after implantation [92-101]. However, a clinical trial comparing high dose systemic methylprednisolone to placebo found no difference in hearing outcomes, although one caveat of the study was that all patients received a dose of dexamethasone to reduce post-operative nausea [102].

Overall, the field of pharmaceuticals to treat hearing loss is evolving at a rapid pace, with some therapies for hair cell or neural regeneration already showing promise in clinical trials for the treatment of deafness with complex etiologies, including hearing loss caused by noise damage, antibiotics and ageing and to improve outcomes with cochlear implantation. Nevertheless, off-target side effects, pharmacokinetic properties and effective cochlear drug delivery strategies that deliver drug in sufficient quantities or over a sufficient time period and without causing further damage to residual cells remain a challenge. Gene therapy is a technique that can overcome some of these issues, as a single intervention can result in lasting outcomes and can be employed as a monogenic therapy for specific genetic causes of hearing loss but also complex etiologies of hearing loss.

3.3 Gene therapy

It is without a doubt that the field of cochlear gene therapy is at an inflection point. Over the last few years, tremendous advances have been made driven mainly by improvements in genetic screening and sequencing technologies, identification of novel gene therapy delivery systems and optimizations of surgical approaches and routes for gene delivery into the cochlea. Gene therapy offers the opportunity to treat both monogenic and complex etiology hearing loss types.

Gene therapy is the introduction of normal genes into the cell, most often via an inactivated viral vector, either to replace a defective gene or to augment gene expression to treat a disorder. The cochlea is well-suited to localised gene therapy as it is surgically accessible, fluid-filled, encased in bone, isolated from other organs and protected from the immune system via the blood-labyrinth barrier [103]. Most studies investigating gene therapy to the cochlea have used adeno-associated virus (AAV) as the gene delivery vector due to its proven safety profile and natural diversity of serotypes which can help target particular cell types [104]. Gene expression can be further localised by delivery of the vector to sub-compartments of the cochlea such as the scala media [105-111]. Injection through the round window membrane or via a cochleostomy drilled through the bony cochlear wall introduces the vector into the perilymph of the scala tympani with diverse gene expression patterns including hair cells and supporting cells of the organ of Corti, marginal cells of the lateral wall and spiral ganglion neurons [112-117]. Cochlear cells can also be efficiently transduced via injection of viral vectors into the semi-circular canals or utricle of the vestibular system, as a strategy to lower the risk of damaging residual cochlear hair cells due to trauma during injection [118-122] .

Inconsistent transduction along the length of the cochlear spiral is a persistent problem encountered following injection into the scala tympani [105,114,117,123,124]. This may be attributed to subtle differences in anatomy and cell morphology between the base and apex of the cochlea [125-127], poor diffusion or flow of the vector following intracochlear injection and loss of the vector to the cochlear aqueduct [128]. The cochlear aqueduct is proximal to the round window membrane and forms a continuous passage with the cerebrospinal fluid and the contralateral cochlea via the subarachnoid space, although it is not always patent in mammals and transduction of cells in the spinal cord and contralateral cochlea is not always observed. Its purpose is to maintain fluid and pressure homeostasis in the cochlea. Injection of a vector can easily disrupt this homeostasis and cause fluid egress from the cochlea, reducing the consistency of viral transduction. A proposed solution to the transduction gradient along the cochlear spiral is to encourage flow through the cochlea by creating an artificial pressure release area distal to the cochlear aqueduct by fenestration of the semi-circular canals. This approach was found to improve transduction in the cochlea [129].

Systemic intravenous delivery would remove the need for traumatic surgical access to the cochlea for gene therapy but not all vectors readily cross the blood-labyrinth barrier. Shibata et al. [130] found systemic intravenous delivery of AAV9 via the temporal vein in neonatal mice robustly transduced spiral ganglion neurons in both cochleae with no impact on hearing thresholds. Transduction of the cerebral cortex, cerebellum and quadriceps' skeletal muscles was also observed, which is consistent with other literature using the same viral vector [131,132]. Unfortunately, successful systemic transduction in mice does not necessarily translate to other models. Studies of AAV-PHP.B, an AAV serotype developed in C57BL/6 mice to be especially efficient at crossing the blood brain barrier and transfecting cells in the brain, was found to offer no improvement over AAV9 in non-human primates and other mouse models [131,133].

Antisense oligonucleotides, which are used as a tool to block gene expression, are also capable of crossing the blood-labyrinth barrier [134,135]. In a mouse model of Usher syndrome, intraperitoneal injection of an antisense oligonucleotide ASO-29 successfully blocked a mutated splice site in a type

1 Usher syndrome gene and restored hearing and vestibular function in the mouse [136-138]. In general, however, the blood-labyrinth barrier presents a significant obstacle for transduction of cochlear cells via systemic injection and the high vector doses required may induce an immune response and other side effects.

3.3.1 Monogenic gene therapy

On average, 1 in 500 newborns have a hearing impairment, with over 50% of these being hereditary in nature. Most causes of genetic deafness have been attributed to monogenic defects. To date, approximately 140 genes have been confirmed to cause hearing loss, with many more that remain to be discovered (https://hereditaryhearingloss.org/). Genetic deafness can be classed as non-syndromic (the only symptom is deafness) or syndromic (accompanied with other symptoms). Non-syndromic autosomal recessive mutations accounts for almost 80% of prelingual (early-onset) inherited deafness, with the most prevalent being a mutation of *GJB2* (a connexin 26 gap junction protein). Syndromic hearing loss makes up the remaining 20% of prelingual genetic deafness and includes Usher syndrome and Jervell and Lange-Nielsen syndrome. In most cases of genetic hearing loss where the mutation is known, gene therapy to replace or augment the defective gene is a viable treatment approach.

The groundwork establishing the feasibility of this approach in restoring hearing function was first successfully demonstrated by Akil and colleagues. They showed that replacement of an absent gene (*VGLUT3*) by delivery of the wild-type gene via AAV1 into congenitally deaf mice led to a near complete reversal of their structural and functional hearing loss phenotype [124]. Since then, there have been a plethora of studies showing rescue of hearing loss caused by monogenic defects after treatment with gene replacement or editing therapies in animal models [111,114,115,117,139-142]. We refer the readers to some recently published reviews highlighting some of the key findings from this work [143,144].

Despite the successes of these pre-clinical studies, several safety and efficacy considerations need to be made. Firstly, most studies showed positive outcomes when treatment was administered to mice at the neonatal stage. Given that the human inner ear completes development *in utero*, further investigation of the critical treatment window and efficacy in mature ears remain pending. Next, understanding the impact of virally mediated ectopic expressions is also necessary, especially when strong but ubiquitous promoters are used (e.g., CMV or CBA). At least from short term studies (<3 months), no adverse effects have been reported in terms of hearing function or cochlear morphology upon the use of these ubiquitous promoters in the mouse cochlea [111,115]. However, it will be interesting to determine if the application of cell-specific promoters improve safety and treatment outcomes. Another crucial consideration is the longevity of the treatment effect. Some studies have indicated only a transient treatment effect lasting from ~7 weeks to 6 months in mouse models [139]. The mechanisms underlying this loss in treatment effect remain unclear but may likely be caused by ongoing cellular degeneration. Along with efficacy, safety outcomes including the pharmacology and toxicological parameters post-overexpressing or silencing a gene of interest need to be thoroughly examined.

Testing efficacy and safety in larger animal models such as non-human primates (Section 3.3.3) and application of cochlear organoids using human pluripotent stem cells (Section 3.1) will provide valuable data for a smooth transition of this technology to clinic. Given the challenges and the broad potential of this technology, there has recently been significant investment into this research. Companies like Applied Genetic Technologies Corporation, Akouos, Rescue Hearing, Novartis, and Decibel Therapeutics are actively involved in preclinical/clinical trials in this research space.

3.3.2 Complex etiology gene therapy

More common forms of hearing loss, including presbycusis, noise-damage, infection, and ototoxicity, can also be targeted using gene therapy. This approach involves inducing hair cell regeneration by activating transdifferentiation of residual non-sensory supporting cells into hair cells in the deaf cochlea. The basic helix-loop-helix transcription factor Atoh1 (also known as Math1) is regarded the master transcription factor required for hair cell development, with embryonic loss of Atoh1 leading to a complete loss of cochlear and vestibular hair cells [145]. Two of the earliest breakthrough studies showed that adenovirus-mediated overexpression of Atoh1 in supporting cells of deaf adult guinea pigs induced the formation of new, ectopic hair cells and promoted some hearing improvement [146,147]. These results initiated a cascade of studies aimed at improving hair cell regeneration in the cochlea using Atoh1 gene therapy, with some reports of mixed or variable outcomes in terms of the extent of hearing recovery post-treatment in animal models [146-150]. Nevertheless, a clinical trial was initiated by Novartis in 2014 to assess the potential of Atoh1 gene therapy (CGF166- ClinicalTrials.gov Identifier: NCT02132130), the first clinical trial to assess adenoviral gene therapy for hearing loss treatment. The clinical trial concluded in December 2019, but the findings from this trial have yet to be revealed to the best of our knowledge.

The current consensus is that overexpression of Atoh1 in supporting cells alone is insufficient in promoting effective hair cell regeneration. There is evidence indicating that overexpression of a multi-factor hair cell gene combination enhances regeneration, but to date, no improvements in hearing recovery in adult mice has been demonstrated [151-154]. An alternate approach is to prime adult supporting cells to a "younger" developmental state either by activating pluripotent genes or targeting epigenetic factors, thus making them more conducive to regeneration. Of note, a recent study showed that delivery of a combination of pluripotent genes (Oct4, Sox2 and Klf4) to adult mouse retinal ganglion cells completely reversed vision loss in mouse models of glaucoma and aged mice, mediated through epigenetic mechanisms [155].

Although inducing hair cell regeneration is considered to be the ultimate remedy for hearing loss and despite significant progress being made, this pursuit has proven to be very challenging. The success of the therapy relies on the degenerative status of the cochlea, whereby preservation of supporting cells and structural integrity of the sensory epithelium is vital. The therapy may also not be suitable for all types of hearing loss. Preservation and regrowth of hair cell synapses and of spiral ganglion neurons is another important goal for hearing restoration. Viral vectors have been used to deliver genes for neurotrophic factors into mesothelial cells lining the cochlear scalae or supporting cells in the organ of Corti with evidence of regrowth of spiral ganglion neuron processes towards the cells releasing the neurotrophic factors [105,156]. The survival of spiral ganglion neurons was more sustained compared to drug-eluting delivery systems [157]. A virus-free method of introducing the neurotrophic factors is also being considered. Using electrical stimulation from the CI in a novel way, a cDNA encoding gene for brain-derived neurotrophic factor was delivered to the guinea pig cochlea and transfected into mesenchymal cells lining the cochlear scalae via close-field electroporation, initiating regrowth of peripheral fibres of spiral ganglion neurons [158]. This study is now the subject of a clinical trial based (anzctr.org.au Identifier: ACTRN12618001556235). While there is clear benefit of preserving the spiral ganglion neural population, neurotrophin therapy also has the potential to disrupt normal innervation of hair cells and residual hearing [159], thus localisation of neurotrophin gene expression to the area of damage may be warranted. There is also the potential for the efficacy of genetic therapies to wane over time due to epigenetic changes or loss of the modified cell [158].

3.3.3 Non-human primate animal models

Mouse models of hearing loss are a valuable tool to study gene therapy and gene editing approaches to restore hearing. However, the large differences in size and anatomy between mouse and human cochleae preclude the generalisation of findings from mouse to human. Studies in non-human primate models offer a more relevant insight into gene expression patterns and safety of delivery of viral vectors to the cochlea. Modelling the larger injection volume that would be required for gene therapy in humans, a 10-30 μL saline injection in rhesus macaques was found to have little negative impact on hearing thresholds and vestibular function [160]. A surgical technique for viral vector injection was developed first in cadaveric, and then live, rhesus macaques for optimised visualisation of the round window membrane, which is the most likely route of injection for human hearing loss therapy [161]. Studies investigating viral tropism and efficacy of transduction in non-human primates have validated findings in mice of highly efficient transduction of inner and outer hair cells by viral vectors such as Anc80L65 [161], AAV9-PHP.B [115,162], and AAV-S [163] and indicated that the procedure is well tolerated and safe. These studies are encouraging for translational studies for hearing therapies, however, variability in transduction [162], failed transduction [115,161] and loss of hearing [163] were reported in some studies, suggesting that further procedure optimisation is required, and there have not been any hearing restoration efficacy studies in non-human primates to date.

3.3.4 Gene editing

While gene therapy introduces normal copies of genes into the cell, gene editing approaches can be used for targeted gene disruption or repair of mutations to restore gene function. Using a transgenic mouse in which hair cells express a fluorescent reporter gene, targeted disruption of the reporter gene was demonstrated for the first time in up to 20% of outer hair cells using a Cas9:sgRNA complex [164]. A nuclease-free base editing strategy was then applied in neonatal mice to disrupt the post-translational phosphorylation of the β-catenin gene, thus activating the Wnt signalling pathway and promoting cellular reprogramming of supporting cells to a hair cell fate [165]. Later, disruption of the *Tmc1* gene (transmembrane channel-like 1) containing a dominant mutation in the *Beethoven* mouse model of hearing loss was reported, wherein Cas9-guide RNAs in lipid complexes or an AAV successfully targeted and knocked down the mutated gene in hair cells while leaving normal genes unaffected. Treated mice had better hearing thresholds and higher hair cell survival than control mice, these effects remaining stable for up to a year [166,167]. But recessive point mutations require correction rather than knockdown. Using Baringo mice which have a recessive loss-of-function point mutation in the *Tmc1* gene, researchers injected AAV-packaged base editing tools and demonstrated repair of the mutation with approximately 50% efficiency but in a low proportion of hair cells [168,169]. Gene editing may also find application for acquired hearing loss. Mice with CRISPR/Cas9-based knockdown of the *HtrA2* gene, that is up-regulated following exposure to aminoglycosides, exhibited protection from neomycin-induced apoptosis up to 8 weeks [170]. Gene editing techniques have enormous potential to treat sensorineural hearing loss, with the major challenges being efficient targeting of the guide RNAs and editing tools to the correct cells along the whole length of the cochlea, timing of the therapy before extensive degeneration occurs and specificity of guide RNAs to target mutated alleles over wild-type alleles.

Looking ahead, a faster route to improve hearing outcomes for patients may be to improve the function of the CI, for example, improving residual hearing, the nerve-electrode interface or the development of paradigm shifting technologies such as optical CIs.

3.4 Optogenetic/optical cochlear implants

Because light can be focussed, through lenses and other techniques, light (or optical) stimulation allows stimuli to be shaped and directed towards target tissues. Through careful design, adjacent light sources (optrodes) can be focused to avoid overlap, permitting more numerous, narrow independent channels and simultaneous stimulation that may overcome the limitations of electrical stimulation. There are several proposed methods of using light to stimulate neural tissue: infrared stimulation, photothermal stimulation, photovoltaic stimulation, photochemical tools, and optogenetic methods. Regarding hearing loss, infrared stimulation and optogenetic methods have been widely investigated in the cochlea.

Infrared light can be used to directly stimulate neural tissue in a technique known as infrared neural stimulation (INS) [171]. Izzo et al. [172] were the first to demonstrate the feasibility of INS in the cochlea by evoking auditory brainstem responses in gerbils. The exact mechanism of INS in the cochlea remains disputed, with some results indicating that part of the response is mediated by hair cells.

Optogenetics refers to the technique of using light to modulate cells, typically neurons, that have been genetically modified to be sensitive to light via "optogenetic actuators" such as type I opsins (i.e., microbial opsins). Type II opsins, the vertebrate counterpart of microbial opsins, are less commonly used as optogenetic actuators and have not been investigated for hearing restoration. Several studies have demonstrated optogenetics as an effective tool for reducing spread of activation in the cochlea (**Figure 2B**) in a diverse range of animal models.

3.4.1 Opsins

Type I opsins are light gated ion channels (or transporters) – i.e., they facilitate the flow of charged ions across the plasma membrane of a cell in response to light. Natural or engineered variants of the microbial opsin channelrhodopsin-2 have produced a diversity of ion channels with different light sensitivities, kinetics, ion selectivity and activation wavelengths. As blue light exhibits greater scattering in tissue compared to red light, there is a preference for red light activated opsins in deep tissue applications. Some opsins have been discovered with peak activation wavelengths in the red spectrum or have been engineered to exhibit a red-shifted peak activation wavelength [173,174]. **Table 1** lists opsins that have been used in optical cochlear implant studies to date.

3.4.2 Optical Cochlear Implants (oCIs)

Optogenetic stimulation was first demonstrated in the mouse cochlea by Hernandez et al. [175], using a fibre-coupled laser inserted through the round window and directed towards auditory neurons expressing the opsin ChR2. Optogenetic stimulation resulted in higher spatial precision than monopolar electrical stimulation, as measured from inferior colliculus recordings. Similar results were observed by Dieter et al. [176] in the gerbil cochlea, with optogenetic stimulation outperforming both monopolar and bipolar electrical stimulation. However, both Wrobel et al. [177] and Thompson et al. [178] observed substantial spread of light in the cochlea using the same light delivery technique. In both studies, brainstem or cortical neurons corresponding to apical positions of the cochlea could be activated with a basally positioned optical fibre. Simulations indicate that this is a consequence of light penetration to apical regions of the cochlea at high powers, emphasising the importance of designing robust light delivery devices for the complex anatomy of the cochlea.

Optical cochlear implants can be divided into two categories according to the means of light delivery. One method is to implant light emitters into the scala tympani, to directly stimulate spiral ganglion neurons. Such oCIs use either commercially available [179] or custom fabricated [180,181] light-emitting diodes (LEDs) arranged into an array in a similar fashion to the electrodes of an electrical CI. Recent developments in gallium nitride (GaN) µLED technology have allowed for production of LEDs on the scale of tens of micrometres that are biocompatible [180]. oCIs comprised of these µLEDs inserted into the gerbil cochlea have been found to result in a higher spatial precision than that of a fibre-coupled laser, even approaching that of normal acoustic tone responses [181]. Although LEDs emit light more broadly than a fibre-coupled laser, these results can be at least partially explained when considering the closer positioning of the µLEDs to spiral ganglion neurons. Measures of localised heating around the µLED arrays in agarose indicate the heat they generate is unlikely to cause damage to the delicate tissues of the cochlea when using short light pulses [179,180], however, further studies regarding chronic implantation are needed.

Alternatively, the light emitters of oCIs can be external to the cochlea, coupled to waveguides such as optical fibres that are inserted into the scala tympani. Consequently, these devices are unlikely to cause heating damage to cochlear tissues but are substantially less efficient due to high losses at the coupling interface [182,183]. Wrobel et al. [177] chronically implanted a single channel waveguide into the cochleae of gerbils and successfully demonstrated optical responses over several weeks. Unfortunately, the histological response to oCI implantation and its effects were not investigated in this study. Overall, there is a lack of research into the long-term safety, temporal precision, and spatial precision of oCIs, which may be impacted by factors such as fibrosis, bone formation and/or damage following implantation and long-term use.

Although the spatial precision of optogenetic-based stimulation strategies has been shown to outperform electrical stimulation, temporal precision is inferior. Electrical stimulation has been shown to achieve a spike probability greater than 95% at 1000 pps in normal hearing cats, and a spike probability of 100% at 400 pps in chronic deafened cats [184]. In contrast, using even the fastest opsin to date, optogenetic stimulation achieves a typical firing probability of only 60% in auditory neurons at a stimulation rate of 100 pps [185]. Similarly, Thompson et al. [178] used multichannel recordings from the inferior colliculus to measure the maximum stimulation rate to optical and electrical stimulation in mice expressing the relatively slow opsin ChR2-H134R in auditory neurons. Electrical stimulation outperformed optical stimulation, however the addition of subthreshold electrical stimulation to optical stimulation improved the maximum stimulation rate by more than 2-fold. When combined with rapid opsins, such a technique may achieve maximum stimulation rates better approximating those of contemporary cochlear implants without sacrificing the high spatial precision of optical stimulation.

The limited temporal precision of optogenetic stimulation is believed to result from two factors. Firstly, the off-kinetics (commonly described as the time constant, τ_{off}) of many opsins are slow, resulting in a delayed return to resting potential after light stimulation (**Table 1**). Research to engineer opsins and improve their kinetics have in turn improved the maximum stimulation rates at the auditory nerve level. Mager et al. [174] engineered two variants from Chrimson; fast (f-) Chrimson and very fast (vf-) Chrimson with τ_{off} of 3 ms and 1.6 ms respectively. Accordingly, vf-Chrimson demonstrates sizeable auditory nerve compound action potentials up to 500 Hz, compared to 200 Hz for the slower f-Chrimson [174,186]. Conversely, these differences are not observed when recording from single spiral ganglion neurons. Rather, the spike probability is similar across f-Chrimson, vf-Chrimson, and Chronos (τ_{off} = 0.7 ms), falling to a spike probability of 50% around 200 pps [174,185,186]. Further research into engineering of opsins may lead to opsins

closely approaching the kinetics needed for the rapid stimulation typically used in contemporary electrical CIs.

Secondly, poor trafficking or insufficient expression of opsins may also limit the temporal precision of optogenetic stimulation. Opsins must be expressed in sufficient quantity and successfully trafficked to the plasma membrane to elicit enough depolarization in response to light to trigger an action potential. Keppeler et al. [185] showed that improving the trafficking of Chronos in spiral ganglion neurons led to significant improvements in the maximum stimulation rate that could elicit an ABR response. This was not seen in the case of vf-Chrimson, suggesting that other factors such as the percentage of transduced neurons may have a more significant effect on outcomes [186]. Indeed, successful transduction of spiral ganglion neurons is critical for the use of optogenetics as a treatment for hearing loss, but is highly dependent on a number of factors, as reviewed below in Section 3.4.3.

3.4.3 Genetic modification of spiral ganglion neurons for oCIs

An optogenetic CI platform requires that the spiral ganglion neurons are permanently genetically modified with an optogenetic actuator. However, transduction of spiral ganglion neurons in animal models has proven to be highly variable, with several factors influencing outcomes for oCIs. Injection of viral vectors into the cochlear perilymphatic fluid via the round window membrane resulted in reliable transduction in a high proportion of spiral ganglion neurons in neonatal mice [174,185,187-189] (**Figure 4**), but was much less efficient in adult mice and gerbils, with poor reproducibility and poor expression in basal turn neurons [177,189,190]. To achieve transduction of spiral ganglion neurons in mature gerbils, a pressure injection directly into the spiral ganglion enabled expression in the order of 25% of spiral ganglion neurons [176,177,190]. Furthermore, up to 10% of spiral ganglion neurons were transduced when injecting the viral vector via the semi-circular canals, but with much lower efficiency compared to hair cells [118]. Conversely, in the adult cynomolgus monkey, AAV injection into the scala tympani of the cochlea via the round window membrane transduced spiral ganglion neurons throughout the cochlea [162], lending hope that this potentially translatable technique could be applied prior to cochlear implantation in humans to genetically modify the spiral ganglion neurons with a light-sensitive optogenetic actuator. In animal models, viral-mediated expression of the opsin is long term, with no evidence of declining expression observed in studies to date [174,177,187]. Optical responses have been detected with as few as 6% of spiral ganglion neurons transduced with opsins, but the optical activation threshold decreases with increasing transduction rates [189,190]. Higher opsin expression levels within individual spiral ganglion neurons also correlates with optical excitability, but high expression levels may negatively impact on intrinsic firing properties of the neurons [191].

Further efficacy of optogenetics for sensory restoration can be found in studies in the retina for restoration of vision. Preclinical proof of concept was obtained in mice in which AAV-mediated expression of opsins in retinal neurons restored visually-evoked responses in the visual cortex with high spatiotemporal resolution as well as pattern recognition [192-200]. The high light intensities required for activation led to retinal phototoxicity concerns, driving the exploration of ectopically expressing the highly sensitive opsins that are intrinsic to the photosensors of the eye, despite their slower kinetics, such as rhodopsin, melanopsin, and cone opsin [195,201-205], as well as alternative promoters to drive strong opsin expression, thus lowering activation thresholds [206].

There are several early-stage clinical trials in progress, sponsored by Allergan/Retrosense (NCT02556736), GenSight Biologics (NCT03326336) and Applied Genetic Technologies/Bionic Sight (NCT04278131), and Nanoscope Therapeutics (NCT04945772), that so far have indicated the safety

and tolerability of expressing channelrhodopsin variants in the eye via AAV vectors. All trials have targeted patients with extensive degeneration of the photoreceptors of the retina due to retinitis pigmentosa. Partial recovery of vision has been reported, such as 20-100-fold increase in light sensitivity, detecting light and motion and direction of motion. In one participant, an AAV construct was used for expression of ChrimsonR-tdTomato along with stimulating glasses to amplify the visual stimulus. Partial recovery of vision was achieved such that he was able to perceive and reach for a notebook on the table in front of him 92% of the time, but less successfully for a smaller object [207]. The emerging evidence from these studies bode well for translating optogenetics for neurological applications in humans, but specific clinical trials will be required to ensure similar safety and tolerability applies to the cochlea.

4.0 Conclusion

New therapies targeting regenerative pathways or effecting synaptic repair or neural preservation after hearing loss show promising but variable efficacy in preclinical studies for acquired hearing loss. The challenges for translating the technologies into human clinical trial include safe and effective delivery of the therapeutics to the cochlea and the poor longevity of the outcomes. Likewise, many gene therapy and gene editing therapies in mouse genetic models of hearing loss demonstrate restoration of hearing, but requires early intervention, often prior to birth, and the low targeting efficiency remains an obstacle. Some researchers are considering adjunctive therapies to the cochlear implant to help improve function, such as improving the nerve-electrode interface or optogenetics to enable optical cochlear implant technology for improved spectral selectivity, which present with similar translational hurdles. Further preclinical studies in multiple animal models, including non-human primates, will help overcome some of these barriers and translate the exciting discoveries observed in rodent models into clinical trials.

5.0 Expert Opinion

With no currently approved pharmaceutical intervention for sensorineural hearing loss, management of hearing loss is restricted to hearing aids and implanted neural interface devices such as the CI. While the CI has restored the gift of speech understanding to over 700,000 people world-wide, emerging alternative therapeutic approaches may improve CI outcomes or even reverse hearing loss. Research into pharmaceutical or gene therapies for hearing loss is now relatively mature since the first studies emerged more than 25 years ago, while other approaches such as gene editing are more recent. Some of these studies are now in clinical trial, with promising signs of improvements in hearing thresholds in some cases.

The causes of hearing loss are incredibly diverse, with estimates of over 400 different types of genetic deafness, in addition to environmental damage to the delicate cells of the cochlea. The complex etiologies of hearing loss will make it difficult to apply specific gene therapies and will certainly lead to variable outcomes. Promising studies showing reversal of congenital hearing loss in mouse models will not directly translate to humans because cellular degeneration often begins prior to birth in humans, requiring *in utero* diagnosis and gene therapy. This is not insurmountable with studies showing successful and safe *in utero* delivery of genetic material to the inner ear [208,209]. For acquired hearing loss, the progressive nature of degeneration of sensory cells can result in a flattened, depleted sensory epithelium, a state from which it is difficult to initiate regeneration. But if treated promptly, there is convincing evidence that pharmaceutical manipulation of the Notch and Wnt signalling pathways or epigenetic priming of inner ear progenitors can remove the barriers that prevent natural cell reprogramming in mature cochleae, generating new hair cells and improving hearing in preclinical research and clinical trials. Advances in technologies such as human organoid

drug screening is increasing the rate of drug discovery for repairing or regenerating hair cells and spiral ganglion neurons. Furthermore, the most vulnerable point of the auditory pathway, the synapse between the hair cell and spiral ganglion neuron, can be restored with neurotrophic factors, expanding the application of potential therapies to nearly all etiologies and severities of sensorineural hearing loss. When applied to patients with partial hearing loss, it will be essential that the therapeutic agents are delivered to the cochlea safely, without causing further damage to residual functional sensory cells. This is best achieved via external application to the semi-permeable round window membrane, although it is difficult to consistently achieve high doses in the cochlea, especially near the cochlear apex.

Cochlear anatomy presents many challenges for gene delivery. While the blood-labyrinth barrier precludes systemic delivery in many cases, the fluid filled spiralling scalae are ideal for localised therapies. Reporter gene expression has highlighted that spread beyond the inner ear is rare and that spread to the contralateral ear does not occur in the non-human primate [115]. It is inevitable that the therapeutic agent will spread to the vestibular system, so it will be important to ensure balance is unaffected. Compared to the periphery, cells in the cochlea are relatively protected from systemic immune responses, meaning that a single dose can yield long-term benefits, but pre-screening for anti-AAV antibodies and transient immunosuppression may be necessary to avoid a potential immune response to AAV administration. Reducing variability in gene expression and overcoming limitations of AAV capacity should remain top priorities. Strategies to overcome these limitations include encoding the gene across two vectors and utilising protein recombination to reconstitute the protein [142,210,211].

Optogenetics, the genetic manipulation of neurons to introduce a light responsive actuator, is a powerful tool that is likely to be applied clinically for neural modulation, not just for hearing. Optical CIs have the potential to increase the resolution of neural activation with relatively little risk, especially if the array contains electrodes as well as light emitters, thus retaining the ability to use electrical stimulation when required. The major challenge for oCIs is to match the extraordinary reliability and safety of contemporary CIs, whereby an implanted device is expected to last a lifetime. An optical device relies on permanent opsin expression in spiral ganglion neurons, and dependable encapsulation of any electronic components. Successful uptake of optogenetic technology will, therefore, depend upon demonstration of a step-change in clinical outcomes.

As preclinical research into hearing restoration increases, and as the public continues to gain confidence in gene-based therapies, pharmaceutical or gene therapies will soon be added to the therapeutic and management options for patients presenting with hearing loss.

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Figure and Table legends

Figure 1. Anatomy of hearing. **(A)** A single turn of the cochlea shown in cross section showing the three fluid-filled chambers (scala tympani, scala media and scala vestibuli) that spiral around the central modiolus. The organ of Corti, which houses the sensory inner and outer hair cells, is located on the basilar membrane. Synapsing with the hair cells are the peripheral fibres of the spiral ganglion neurons whose cell bodies are in Rosenthal's canal. 1. Scala vestibuli; 2. Reissner's membrane; 3. Tectorial membrane; 4. Scala media; 5. Inner hair cell; 6. Outer hair cells; 7. Basilar membrane; 8. Spiral ganglion neuron; 9. Rosenthal's canal; 10. Scala tympani. **(B)** The classical ascending auditory pathway from the cochlea to the auditory cortex showing the key brain regions and connections for auditory processing. The axons of spiral ganglion neurons form the cochlear nerve, carrying sound information into the brainstem. Sound information is processed and integrated from both ears as it ascends to the auditory cortex where perception occurs. 1. Auditory cortex; 2. Medial geniculate nucleus; 3. Inferior colliculus; 4. Nucleus of the lateral lemniscus; 5. Superior olivary complex; 6. Cochlear nuclei; 7. Cochlear nerve.

Figure 2. The cochlear implant. **(A)** An array of electrodes is surgically implanted into the scala tympani of the cochlea. Pitch information is conveyed by place coding and temporal envelope modulations. **(B)** The current spread from two active electrodes, or channels, is shown in blue and yellow and the area of interaction between channels is shown in green (but in reality is much broader than depicted here). The neural population in this overlapping region of current receives electrical current from both electrodes. Hence, while cochlear implants may have between 12 and 24 electrodes, the number of independent information channels is lower due to current spread.

Figure 3. Timeline of some key preclinical and clinical studies for the management and treatment of hearing loss. (Corwin and Cotanch, 1988)[45] (Ryals and Rubel, 1988)[46] (Ernfors et al., 1996)[212] (Izumikawa et al., 2005)[146] (Akil et al., 2012)[124] (Chen et al., 2012)[56] (Mizutari et al., 2013)[64] (Hernandez et al., 2014)[175] (Gao et al., 2018)[166] (Gyorgy et al., 2019)[167]

Figure 4. AAV-mediated expression of a light-sensitive channelrhodopsin ion channel (ChR2-H134R) in spiral ganglion neurons of a mouse. **(A)** Injection of the viral vector into the scala tympani resulted in opsin expression throughout the cochlea and in a high proportion of spiral ganglion neurons. **(B)** High power image showing ChR2-positive and negative spiral ganglion neurons. Study details can be found in [189].

Table 1. Type I opsins used in optical cochlear implant research studies. Despite the variety of opsins available, only excitatory channelrhodopsins have been explored in this application.