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# Novel drug delivery systems for inner ear protection and regeneration after hearing loss

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*Background*: A cochlear implant, the only current treatment for restoring auditory perception after severe or profound sensorineural hearing loss (SNHL), works by electrically stimulating spiral ganglion neurons (SGNs). However, gradual degeneration of SGNs associated with SNHL can compromise the efficacy of the device. *Objective*: To review novel drug delivery systems for preserving and/or regenerating sensory cells in the cochlea after SNHL. *Methods*: The effectiveness of traditional cochlear drug delivery systems is compared to newer techniques such as cell, polymer and gene transfer technologies. Special requirements for local drug delivery to the cochlea are discussed, such as protecting residual hearing and site-specific drug delivery for cell preservation and regeneration. *Results/conclusions*: Drug delivery systems with the potential for immediate clinical translation, as well as those that will contribute to the future of hearing preservation or cochlear cellular regeneration, are identified.

**Keywords:** cell-based therapy, conducting polymer, cochlear implant, dexamethasone, gene transfer, hair cell, hearing protection, neurotrophin, polypyrrole, regeneration, sensorineural hearing loss, spiral ganglion neuron

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## 1. Introduction

The World Health Organisation estimates that 278 million people have a disabling hearing impairment, with this number increasing every year as the population grows and ages [1]. Resulting social and economic implications include delayed learning, speech and language development in children, reduced employment opportunities, social isolation and rising medical costs associated with treatments. The causes of hearing loss are numerous: genetic abnormalities, excessive noise, ototoxic substances (e.g., platinum-based chemotherapy drugs such as carboplatin, cisplatin and oxaliplatin, aminoglycoside antibiotics such as streptomycin, neomycin and gentamicin, loop diuretics), infectious diseases, ear infections and ageing. With a sensorineural hearing loss (SNHL), accounting for 90% of all hearing loss, the problem occurs in the sensory cells of the inner ear (hair cells) and/or the spiral ganglion neurons (SGNs) that transmit the auditory information to the brain. Cochlear implantation is the only treatment currently available for severe to profound SNHL, overcoming the loss of hair cells by directly stimulating SGNs with electrical pulses. Cochlear implants therefore rely on a substantial surviving population of healthy SGNs for sound to be transmitted to the brain.

In most forms of SNHL, damaged hair cells and their supporting cells undergo apoptosis and are permanently lost, as they do not naturally regenerate in mammals. Sensory hair cells and supporting cells normally provide SGNs with a continual source of neurotrophins [2,3], hence damage or loss of these cells is considered to be a major factor leading to the secondary degeneration of SGNs [4-6], potentially

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reducing the efficacy of cochlear implantation. Opportunities for preventing or reversing hearing loss therefore lie in the preservation or regeneration of hair cells and SGNs. Compared to other organs in the body, the cochlea is relatively isolated, making it an ideal candidate for localized drug delivery and avoiding the potential side effects of drugs that are prevalent with systemic administration. Localised drug delivery would increase the effectiveness of the drug and overcome the blood–cochlea barrier that prevents drugs of a certain size or charge from entering the cochlea from the bloodstream. This review will discuss current and experimental local drug delivery systems that target hair cells and SGNs of the cochlea, including the emerging therapeutic options of cell, gene and polymer technologies, for preventing damage, preserving or regenerating these cells following SNHL.

## 2. The role of neurotrophins in preserving spiral ganglion neurons following sensorineural hearing loss

The neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) are produced and released by hair cells in the sensory epithelium of the cochlea [7-11]. Loss of hair cells therefore disrupts the normal supply of neurotrophins and initiates secondary degeneration of SGNs. SGN degeneration is characterized by demyelination and retraction of peripheral dendrites within the osseous spiral lamina (OSL). SGN cell bodies in Rosenthal's canal shrink and ultimately undergo apoptotic cell death (**Figure 1**) [12-14]. Degeneration of SGNs is progressive over time [14] and is likely to be a key factor limiting the effectiveness of the cochlear implant. Therefore, there is considerable interest in strategies to replace lost neurotrophic support to prevent deafness-associated SGN degeneration and instigate regeneration of damaged peripheral dendrites.

One of the first experimental neurotrophin delivery systems for the cochlea was a slow-release device known as the mini-osmotic pump [15]. Pumps were tested over treatment periods ranging from days to weeks in guinea pigs (GPs) deafened with aminoglycosides. The mini-osmotic pump delivery system consists of a drug reservoir that uses osmotic pressure for controlled slow-rate release of the drug and a cannula that is directed into the cochlea, usually the scala tympani. BDNF and NT3 were most commonly delivered [16-23], but other growth factors included glial cell line derived neurotrophic factor (GDNF) [24], acidic fibroblast growth factor (FGF) [25] and the neuronal cytokine ciliary-derived neurotrophic factor (CNTF) [26,27], administered alone or in various combinations. A large body of evidence now indicates that neurotrophic factors promote potent SGN survival when administered continuously to the cochlea shortly after SNHL [16-18,28,29]. Neurotrophin infusion into the cochlea via a mini-osmotic pump was also reported to rescue SGNs in experiments where there was a delay between the onset of deafness and treatment, modelled on the clinical situation where considerable SGN degeneration has usually occurred before intervention [20,22,27,30]. Furthermore, the peripheral dendrites of SGNs were larger, longer, more numerous and exhibited less demyelination in neurotrophin-treated cochleae compared to untreated cochleae [22]. These results provide encouragement that neurotrophins may be used as a therapy to rescue and protect residual auditory nerves in patients who have been deaf for a considerable period of time.

Neurotrophins are important for the formation of normal innervations in the cochlea during development and evidence is beginning to mount that they also play a key role in promoting regeneration of SGN peripheral dendrites in the adult cochlea following SNHL [16-18,22,25]. However, regenerating dendrites tended not to follow their original growth pattern, due to the significant degeneration of the organ of Corti, with complete loss of hair cells and supporting cells. Instead, resprouting dendrites were observed in the region of the inner sulcus [22,25], on the basilar membrane [22], within the osseous spiral lamina [22] and in the scala tympani [16,18,25]. Whilst the presence of resprouting auditory neurons offers promise to repair the damaged auditory system, there are important experimental questions to address and significant obstacles to overcome. For example, extensive uncontrolled resprouting of auditory neurons may interfere with the cochleotopic organisation of the cochlea. Such an outcome may reduce the electrode-specificity of a cochlear implant as a consequence of a greater number of auditory neurons being activated by multiple electrodes. Furthermore, any future therapy that aims to 'reconnect' neurons with replaced sensory cells (see Sections 6.5 and 7.2 below) will need to control dendrite resprouting in order to maintain tonotopic organisation. This may be achieved by localised or cell-specific drug delivery in order to provide guidance cues for regenerating dendrites in addition to overall survival signals.

## 3. Neurotrophins and intra-cochlear electrical stimulation

The future use of neurotrophins for maintaining SGN survival and promoting dendritic regrowth in cochlear implant recipients will need to consider the effect of combining intracochlear electrical stimulation with neurotrophin treatment, a strategy explored by very few studies. Kanzaki *et al.* [31] used a viral technique to transfect cochlear cells so that they produced neurotrophins and combined this with intracochlear electrical stimulation. Shepherd and colleagues [32,33] used an intracochlear electrode with a built-in neurotrophin delivery system connected to a mini-osmotic pump. These studies demonstrated that chronic electrical stimulation combined with neurotrophin treatment produced greater SGN survival after SNHL than either treatment alone [31-33], together with the functional advantage of reduced thresholds for electrically-evoked auditory brainstem activity [32,33].

Interactions between neurotrophins and neural activity have been known for some time. One possible explanation

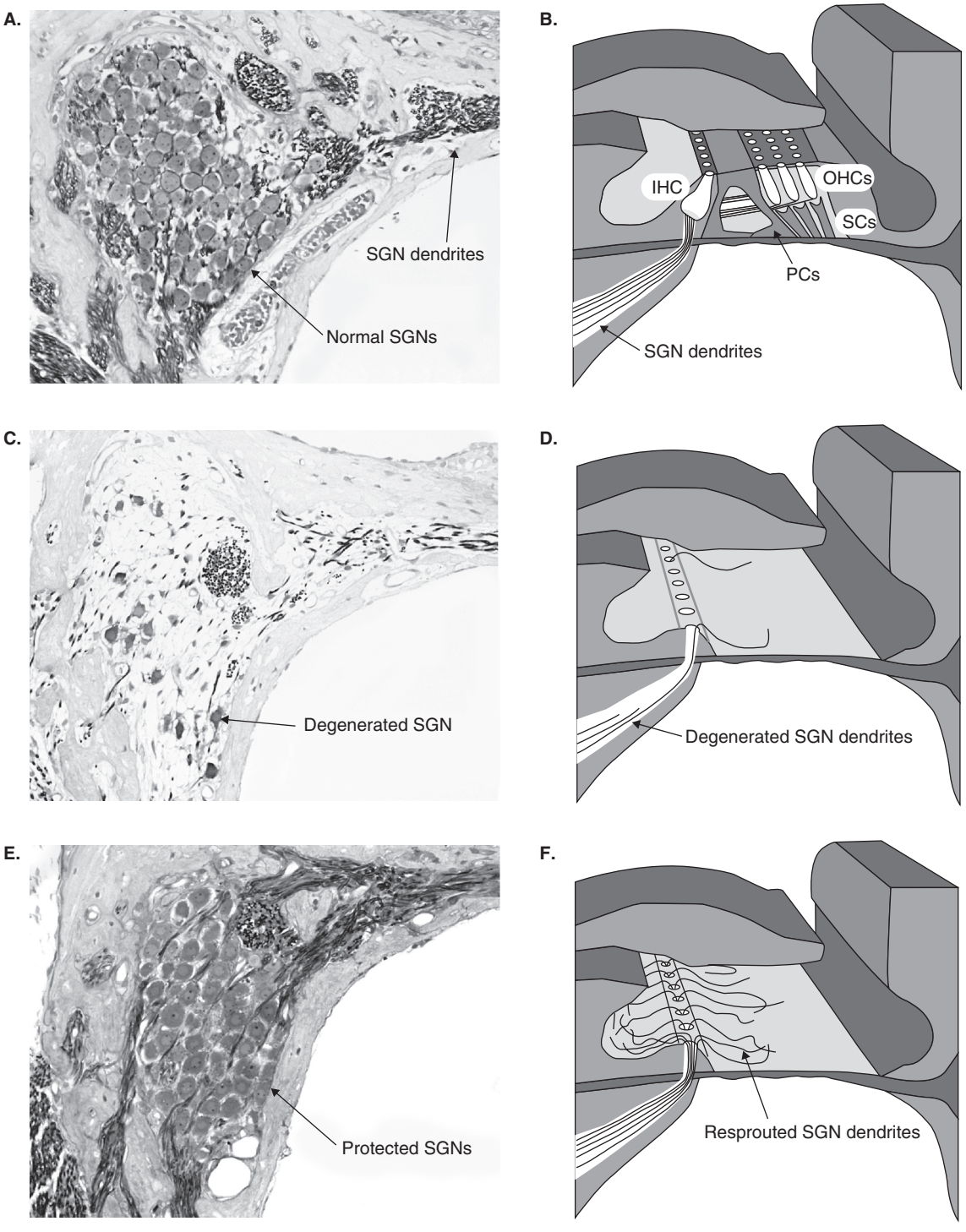


**Figure 1. Degeneration of SGNs after SNHL and the role of neurotrophins in SGN protection and dendritic resprouting.** **A.** Mid-modiolar section of a normal GP cochlea depicting the SGN cell bodies and their peripheral dendrites. **B.** The dendrites project towards and innervate the sensory inner hair cells (IHC) and outer hair cells (OHCs). Supporting cells (SCs) and pillar cells (PCs) provide structural support for the hair cells and are collectively known as the organ of Corti. **C** and **D.** Following an aminoglycoside-induced SNHL that destroys the cellular structure of the organ of Corti, there is significant secondary degeneration and death of the SGNs and their peripheral dendrites. **E** and **F.** Delivery of neurotrophins to the cochlea following the SNHL can protect the SGNs from degeneration and promote resprouting of the peripheral dendrites. However, resprouted dendrites no longer have sensory targets to innervate.

for these observations is that depolarisation changes the responsiveness of neurons to the neurotrophin and the efficiency of cellular processes mediated by neurotrophins. For example, cortical neurons that were depolarised by application of $K^+$ upregulated the expression of full-length TrkB receptors (the receptor for BDNF) in their peripheral processes [34]. Electrical stimulation also enhanced the responsiveness of hippocampal neurons to BDNF by facilitating the movement of TrkB receptors from intracellular pools to the membrane and facilitating the internalization of the TrkB/BDNF receptor complex [35].

It is known that neurotrophin treatment alone only elicits SGN survival and resprouting for the duration of the treatment. SGNs continue to degenerate once neurotrophin delivery ceases, suggesting that a constant supply of neurotrophins is necessary to maintain SGN survival over the longer term [19]. An interesting finding from the study by Shepherd *et al.* 2007 [33] in which neurotrophin delivery was combined with chronic intra-cochlear electrical stimulation was that the continuation of electrical stimulation following cessation of neurotrophin treatment prolonged SGN survival beyond the period of neurotrophin treatment. This has immediate clinical implications for cochlear implant surgery in which short-term neurotrophin delivery to the inner ear may help to maximise the number and health of SGNs for chronic stimulation by the cochlear implant. However, the use of electrical stimulation alone has had mixed results in terms of SGN preservation after SNHL with some reports showing SGN protection and other studies observing no effect on SGN survival [36-38]. Differences may have arisen due to the method of induction of SNHL, type and duration of electrical stimulation and the animal model. It is therefore unclear how long these extended SGN survival effects would last after cessation of neurotrophic support.

While mini-osmotic pumps provide continual drug delivery over the course of weeks, they cannot be used over a patient's lifetime due to the need for frequent replenishment of the drug reservoir and the risk of infection that this poses to the cochlea. There is now an impetus to explore new methods of clinically viable, sustainable and specific neurotrophin delivery to the cochlea for maintaining SGN survival after SNHL. One option that is most immediately applicable to the clinic is the non-traumatic application of drugs to the cochlea via the round window membrane (Section 4). More advanced techniques are also rapidly emerging, including conducting polymer, gene and cell-based therapies (Sections 5 – 7) for longer term or more site-specific drug delivery for hearing protection and regeneration.

## 4. Non-traumatic drug delivery to the cochlea via the round window membrane

One of the simplest and least traumatic methods of applying drugs to the cochlea is to place the drug on the surgically accessible round window membrane, often via a polymer or a hydrogel, allowing the drug to diffuse through the membrane into the perilymphatic fluid of the scala tympani without the need to perforate the cochlea. The round window approach is known to lead to much higher intracochlear drug concentrations than systemic drug delivery [39], hence promoting optimal outcomes in the cochlea while avoiding side effects in other organs. Not every drug is able to diffuse through the round window membrane. However, many drugs of interest have been demonstrated to diffuse across the membrane, for example antibiotics, glucocorticosteroids such as dexamethasone, liposomal and adenoviral gene transfer vectors, and neurotrophins [40-45]. Placement of biodegradable hydrogels containing BDNF or NT3 on the round window membrane of deafened GPs protected SGNs from degeneration, indicating successful diffusion of the neurotrophin into the cochlear fluids [42,43,46]. However, this type of drug delivery is relatively short-term and is better suited to applications such as hearing protection.

### 4.1 Hearing protection during cochlear implantation

For the best speech perception outcomes after cochlear implantation, it is now recognised that electro-acoustic stimulation (a combination of residual acoustic stimulation and the cochlear implant) gives a richer quality of hearing than either modality alone, especially in the presence of background noise [47-50]. As both cochlear implant design and insertion techniques have improved, it was found that a greater proportion of patients retained some of their residual hearing [51-53], widening the candidacy for cochlear implantation to individuals with significant residual hearing. However, there is still a substantial risk of residual hair cell loss in the operated ear.

In GPs, it was shown that simply creating a small opening in the cochlea caused prolonged loss of hearing thresholds. This was ameliorated by direct infusion of a corticosteroid into the scala tympani [54]. Similarly, it was shown in GPs that cochlear implant insertion trauma was eliminated by continuous application of dexamethasone for 8 days via a mini-osmotic pump beginning on the day of implantation [55]. However, a mini-osmotic pump would require an additional surgical procedure to remove the pump and cannula. Therefore, non-invasive, clinically relevant methods of dexamethasone delivery were investigated, with an emphasis on ensuring the drug is in place prior to electrode insertion, as well as during insertion and for a short period afterwards.

In an experimental model of cochlear implantation in GPs, a miniature cochlear implant electrode was implanted approximately 2.5 mm into the scala tympani of the cochlear basal turn, using methods adapted from clinical surgical approaches [45]. This insertion depth is equivalent to a 'short' basal turn electrode insertion in humans. An immediate mild-to-moderate hearing loss occurred, which was greater in the high frequencies (T0; **Figure 2A**), and improved by approximately 10 dB over the first post-operative week (T1; **Figure 2A**). A permanent mid–high frequency hearing

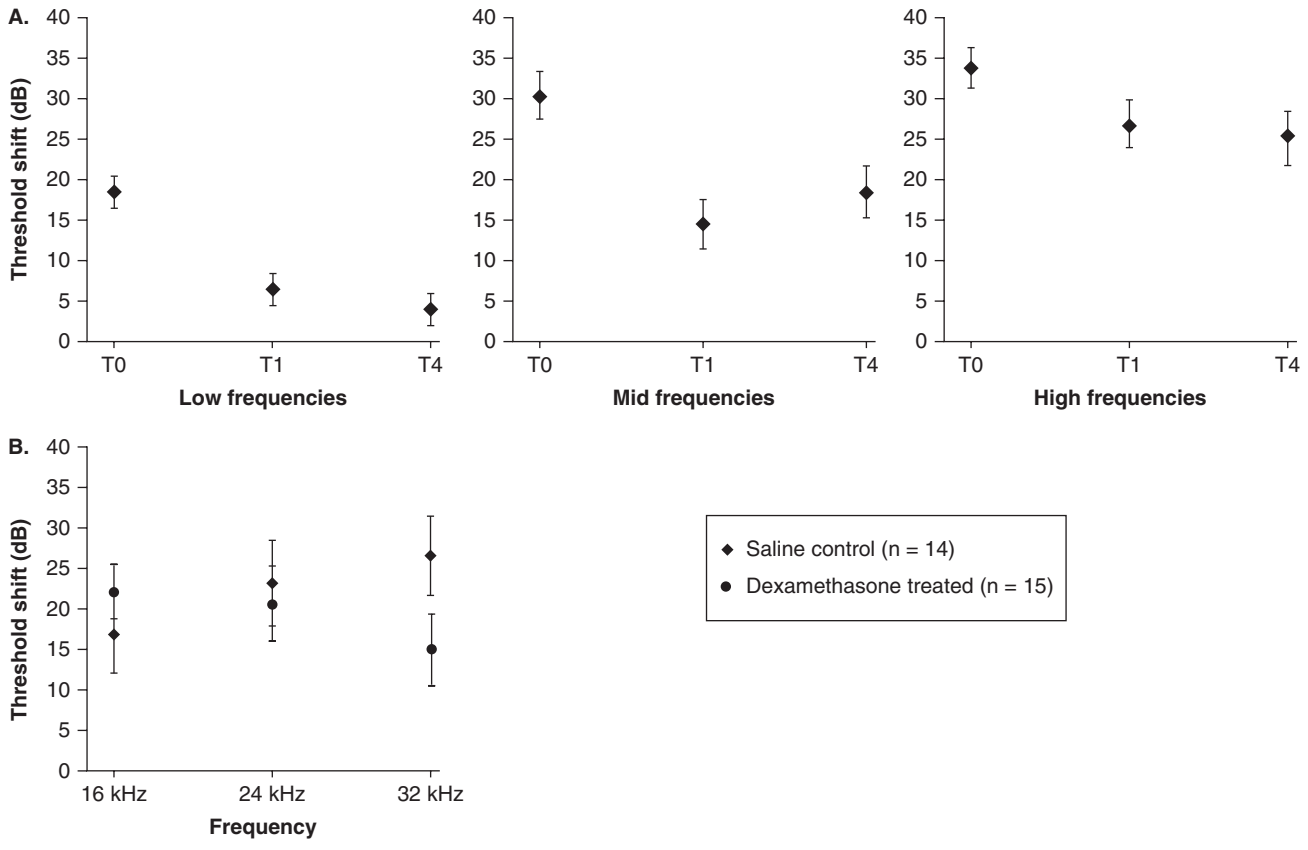


**Figure 2. Delivery of dexamethasone to the round window membrane prior to cochlear implant surgery provides hair cell protection. A.** Auditory brainstem response thresholds to acoustic stimulation were taken before cochlear implantation and compared to auditory brainstem responses immediately after cochlear implantation (T0), 1 week after cochlear implantation (T1) and 4 weeks after cochlear implantation (T4). The results were divided into low frequencies (0.5, 1 and 2 kHz), mid-frequencies (4, 8 and 16 kHz) and high frequencies (24 and 32 kHz). There was an immediate mild–moderate hearing loss from cochlear implantation across all frequencies. Low and mid-frequency hearing recovered over time, but the threshold for high frequency hearing was still raised after 4 weeks. **B.** Application of dexamethasone (circles) to the round window membrane via a polymer 30 min prior to cochlear implantation significantly reduced the threshold shifts resulting from cochlear implantation in the high frequencies where hearing loss was most prominent. Application of saline control treatment (diamonds) had no effect on hearing thresholds. (Error bars indicate the standard error of the mean).

loss followed, with recovery of the lower frequencies (T4; **Figure 2A**). The extent of the high frequency hearing loss was significantly attenuated by the application of dexamethasone to the round window for 30 min prior to cochlear implantation (**Figure 2B**) [45]. Preliminary results from ongoing experiments suggest that hearing preservation is stable for at least three months, and that the degree of preservation is improved by lengthening the time that the dexamethasone is applied to the round window prior to cochlear implantation. Of interest was the finding that application of dexamethasone to the round window membrane reduced the chronic inflammatory tissue response to the cochlear implant, with no detectable multi-cellular giant cell foreign body response to the electrode. Clinically, patients who received intracochlear injection of a corticosteroid at the time of cochlear implantation had decreased post-operative electrode impedances compared to patients who did not receive the steroid [56,57]. This also indicates that corticosteroids can help to reduce the fibrous tissue build-up resulting from the foreign body response to the cochlear implant.

Round window membrane drug delivery is already in clinical use for residual hair cell protection. In cases of sudden onset SNHL, high dose systemic glucocorticosteroids often provide some protection to hair cells and moderately improve hearing thresholds, but not in all cases. If patients fail to respond to systemic treatment, the round window microcatheter [58-60] and Microwick [61-63] are two devices available to locally target the drug to the inner ear via a minor surgical procedure. Both consist of a catheter that is directed invasively through the tympanic membrane towards the round window membrane. Drugs are delivered through the catheter either as self-administered drops in the ear canal or via a continuous pump. Measurable improvements to hearing thresholds (15 – 25 dB) were reported, but long-term outcomes were widely variable.

A major downfall with drug delivery to the round window membrane is the inability to control the drug dose and rate of delivery. Round window drug delivery often leads to a severe base-apex concentration gradient, making it difficult to predict the amount of drug that will be available for cells in different turns of the cochlea [64,65]. Additional uncertainties arise due to natural variability in round window membrane permeability between animals of the same species [66] as well as variability of round window permeability between species and pathologies of the round window membrane that may hinder diffusion of the drug [67]. In some cases, the rate of release of the drug can be controlled by a modification to the polymer or hydrogel, for example by the addition of a substance that alters the affinity of the drug to the substance [68] but release still tends to occur as an initial burst followed by more steady-state release until the material degrades. Furthermore, drug delivery from the round window is not as effective as direct drug delivery to the cochlear fluids. A particular group of polymers known as polypyrroles are known to be electrically conducting as well as drug eluting [69]. If such polymers were applied to the cochlear implant, the eluted drugs could be delivered directly to the SGNs being electrically stimulated, preserving SGNs during and after cochlear implantation.

## 5. Conducting polymers for combined drug delivery and electrical stimulation

For combining short-term delivery of neurotrophins with chronic electrical stimulation from the cochlear implant, it stands to reason that the cochlear implant itself may be used as the drug delivery device. In a study mentioned previously, the cochlear implant was modified to incorporate a hollow tube to connect to a mini-osmotic pump [31]. While suitable in experimental animals, there are serious issues associated with implant designs that incorporate narrow spaces where bacteria could evade detection from the immune system. Therefore, other means of drug delivery via the cochlear implant are being investigated for safer drug delivery.

Applying drug-releasing materials to the cochlear implant gives drugs better access to hair cells and SGNs than drug delivery devices placed on the round window membrane and does not require any further surgery than cochlear implantation itself. In one study, an electrode array was coated with a layer of agarose gel containing fibroblasts modified to express BDNF and implanted into GP cochleae after SNHL. SGN survival was observed in the vicinity of the implanted electrode array [70]. However, coating the cochlear implant electrodes is likely to decrease the effectiveness of delivering electrical charge to excite the SGNs. Therefore, materials that are electrically conducting in addition to having drug eluting properties are of special interest for cochlear implant electrode drug delivery applications. One such material is a polymer called polypyrrole (Ppy) which 'grows' or polymerises only on conducting surfaces such as the platinum electrodes of cochlear implants. The electrically conducting properties of Ppy enable normal functioning of a cochlear implant while drugs such as neurotrophins can be incorporated into the structure of the polymer for subsequent release [71]. Specifically, it was discovered that the quantity of neurotrophins stored in 24 μm Ppy coatings on electrodes was compatible with nerve survival and regrowth in the inner ear [72]. Neurotrophin release rate could be controlled by the level of electrical stimulation delivered to the polymer. In the absence of electrical stimulation a relatively slow rate of neurotrophin release was observed. Applying electrical stimulation to levels similar to that used by the cochlear implant was shown to enhance the rate of release of neurotrophins from the polymer [72].

In an *in vitro* assay, it was discovered that SGN survival and neurite outgrowth from SGN explants were greater when Ppy had incorporated NT3 (Ppy/NT3) compared to explants grown on Ppy without neurotrophins [73]. Application of electrical stimulation to Ppy/NT3 further enhanced neurite survival and outgrowth (**Figure 3**) [73]. These are very encouraging results as they indicate that Ppy can store and release neurotrophins without affecting the biological activity of the neurotrophins or the delivery of charge to the auditory nerve. Secondly, they show that the release of neurotrophins can be controlled to achieve extended delivery of neurotrophins. Furthermore, Ppy has been used for the controlled release of other drugs such as dexamethasone [74], with clear implications for protecting hair cells during cochlear implant insertion.

A key issue to overcome with slow-release polymeric drug delivery is the relatively rapid exhaustion of drug supply with respect to the life of the polymer. Covalent attachment of drugs to polymers, thought to provide a more permanent molecular attachment, could be one solution to providing longer-term therapeutic effects from a finite resource. Immobilized nerve growth factor (NGF) triggers intracellular signalling cascades in cells without internalisation of the ligand [75], thus preserving the ligand for further cell stimulation. Neurite outgrowth from a neuronal cell line was increased when exposed to immobilized NGF on a Ppy polymer, and further improved with electrical stimulation (albeit using a constant current rather than the biologically safe biphasic current pulses used in cochlear implants) [76]. However, this technique is not successful for all drugs or ligands as cellular responses to non-internalised ligands tend to be less effective. The possibility remains that with appropriate drug design and immobilization of drugs on conducting carriers such as Ppy, the duration and safety of drug delivery to the inner ear can be enhanced utilising the cochlear implant electrodes.

## 6. Gene transfer

Drug diffusion from polymers placed on the round window or coating the cochlear implant are limited by the eventual

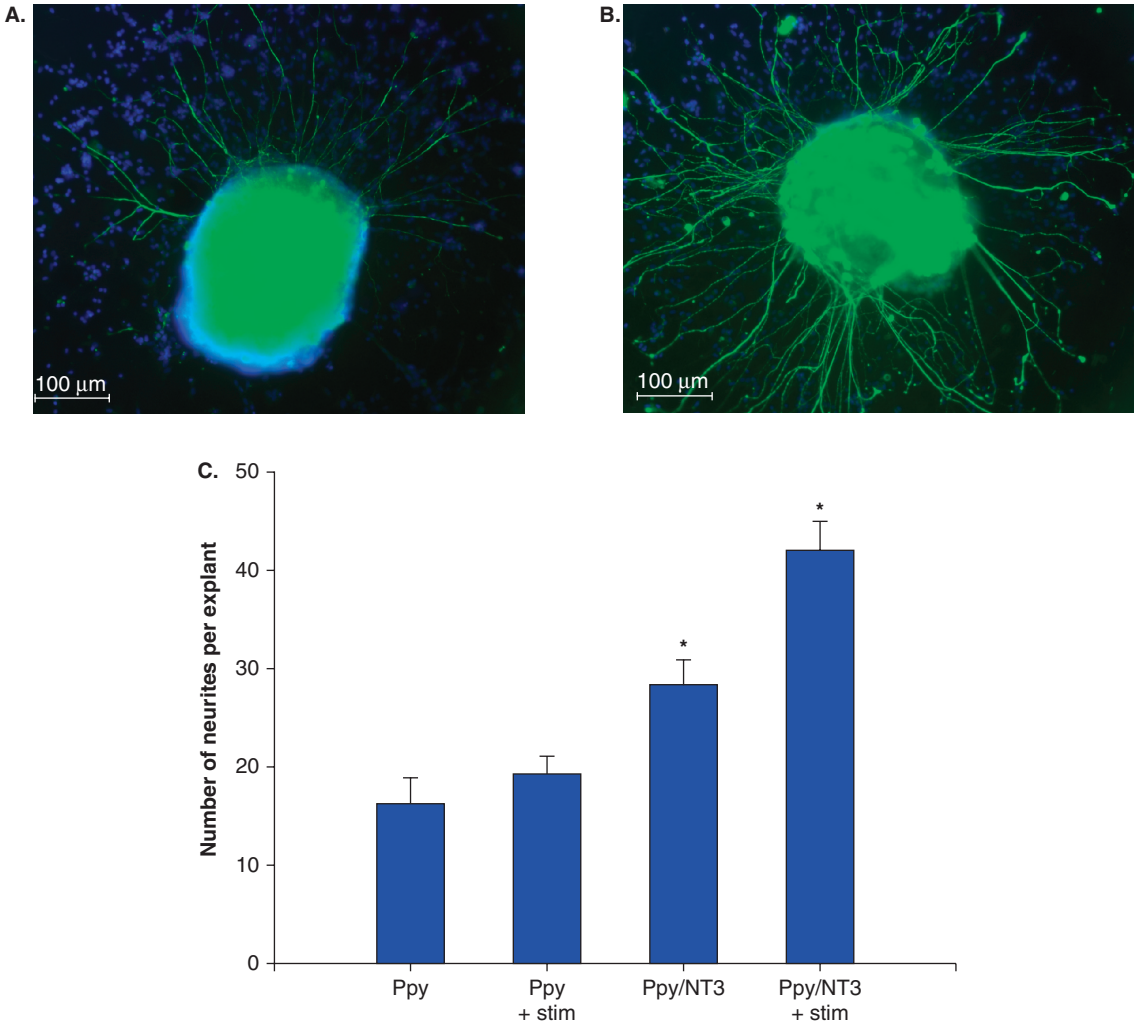


**Figure 3. Controlled release of neurotrophins from Ppy promotes SGN survival and neurite outgrowth.** SGN explant grown on Ppy without incorporated NT3 (**A**) and polypyrrole with incorporated NT3 (**B**) demonstrate that neurite outgrowth is significantly enhanced when the polymer contains NT3 ($p < 0.05$). (**C**) Electrical stimulation of plain Ppy had no effect on neurite outgrowth from explants but electrical stimulation of Ppy/NT3 significantly enhanced SGN neurite outgrowth over and above unstimulated Ppy/NT3 and all other groups ($p < 0.05$; Error bars indicate the standard error of the mean).



depletion of the drug, hence other solutions are required for longer term drug delivery. Gene therapy is being investigated for its potential to provide long-term, stable expression of a therapeutic gene via a single application to the cochlear fluids. Furthermore, the cell-specific expression of genes such as neurotrophins may provide the guidance cues required for controlling the misdirected dendritic regrowth observed after conventional neurotrophin delivery.

### 6.1 Gene transfer vectors

Most work on gene transfer to the inner ear has focused on the use of replication-defective viral vectors such as adenovirus (Ad) [77-81], adeno-associated virus (AAV) [78,79,82,83], herpes simplex virus (HSV) [28,77,84-86] and vaccinia virus [84]. Ad-mediated gene transfer results in relatively short-term gene expression (weeks–months) in non-dividing cells, but can result in toxicity in the infected cell and produce immunogenic reactions [80,87]. The inflammatory response caused by Ad impacts on hair-cell function and hearing thresholds but can be overcome by using glucocorticosteroids such as dexamethasone [88]. Moreover, immunogenic responses to newer generation Ad vectors are much more moderate, allowing transduction of hair cells while preserving hearing thresholds [89]. Toxicity or immunogenicity issues can also be overcome via the use of non-viral gene transfer vectors [41,77,90]. However, these tend to have inferior transduction efficiency compared to viral vectors. AAV confers long-term gene expression in a wide variety of postmitotic cells with low cytotoxicity and immunogenicity. The size of the gene that can be inserted into AAV vectors

is more limited than Ad but this limitation is not an issue for the small neurotrophic factor genes. In contrast, HSV very efficiently and selectively transduces neurons and is often used in studies for this specific purpose [77]. Viral vectors are currently the best experimental tool to evaluate the effect of gene transfer on cochlear cells *in vivo*, with AAV vectors holding the most promise for translational studies in humans.

A key contribution to the study of drug delivery to the inner ear comes from the use of reporter genes in gene transfer vectors and sensitive molecular assays to identify cells that have been transduced. These studies have proven that introduction of vectors into one cochlea leads to transduction of cells in the contralateral cochlea [82,83,91-93], the brain [83,93] and temporal bone marrow spaces [93] via the movement of the vector through the cochlear aqueduct and into the cerebrospinal fluid. The phenomenon is not unique to gene transfer vectors. Delivery of high concentrations of GDNF in one ear of noise-deafened GPs led to SGN protection not only in the injected ear but also in the contralateral ear [94]. Similarly, stem cells injected in one ear were detected in the contralateral ear in mice [95]. This highlights the need for comprehensive safety studies for the delivery of any drug to the inner ear.

### 6.2 Delivery sites for gene transfer vectors

Reporter genes on gene transfer vectors have been used to explore the many possible routes of administration of viral transgenes, although the results can be extended to any type of drug. Different administration routes to the cochlea have been explored with various aims, such as maximizing the number of cells transduced, reducing cochlear toxicity or preserving hearing. Viral transgenes injected directly into the scala tympani distribute broadly, with expression of reporter genes in SGNs, hair cells and supporting cells the organ of Corti, among many other cells and tissues [78,80,86,89,90,96]. Access to the perilymph by diffusion across the round window membrane, thereby preserving cochlear integrity, is possible for Ad and non-viral liposomes but not AAV [41], but is not as efficient as direct injection of the vector into the scala tympani [97]. Although surgically more difficult, injection of gene transfer vehicles directly into the scala media results in efficient transduction of hair cells and supporting cells but risks damage and loss of hair cells around the injection site [87,98,99]. Viral vector access to the cochlea can be achieved via the vestibular organs, helping to reduce trauma to the cochlea and subsequent hearing loss. For example, injection of Ad into the endolymphatic sac resulted in gene expression in the stria vascularis and supporting cells such as Hensen's cells of the organ of Corti in the cochlea [100]. Likewise, the injection of HSV into the utriculus achieved gene transfer in vestibular and cochlear neurons [86]. Vestibular function is generally affected when using vestibular routes, resulting in disabling dizziness and nausea, but this is generally recoverable, as was observed after injection into the posterior semicircular canal of mice [101]. On this note, introduction of transgenes via the vestibular organs is of course a plausible route for treatment of vestibular disorders [92].

### 6.3 Site-specific drug delivery using gene transfer

The main advantage of using gene transfer to deliver therapeutic agents to the inner ear is the ability to target particular cell types. Viral vectors only infect cells that express specific cell surface receptors. For example, cells must express heparan sulfate proteoglycan for receptor-mediated internalization of AAV serotype 2 [102], along with co-receptors $\alpha 5\beta 1$ integrin [103] and FGF receptor-1 [104]. Each serotype has a subtly different infection pattern and thus could be exploited to target particular cells in the cochlea. A comparison of different AAV serotypes for cochlear cell transduction revealed that inner hair cells were most efficiently transduced by AAV serotype 3, while SGNs were most efficiently targeted by serotype 5 [105]. Cell-specific promoters can be used to further target gene expression to certain cell types. Exclusive transduction of hair cells was achieved using the myosin 7a promoter in rats and mice [106,107]. Likewise, a hybrid promoter with sequences from β-actin and cytomegalovirus resulted in efficient hair cell transduction, while a glial fibrillary acidic protein promoter targeted supporting cells in the organ of Corti [108]. As mentioned previously, HSV vectors efficiently target SGNs [77] but other vectors (e.g., AAV) can also be used in conjunction with specific promoters such as neuron-specific enolase for neural transgene expression [79].

### 6.4 Protection of SGNs and hair cells by gene transfer

Experimentally, viral-mediated gene therapy has been successful for the treatment of inner ear disorders. For the preservation of SGNs after SNHL, BDNF gene transfer via HSV, AAV or Ad in ototoxically deafened GPs resulted in greater SGN survival compared to contralateral and control cochleae [28,109,110]. Gene transfer of GDNF at the time of or before gentamicin ototoxic insult protected hair cells and maintained hearing thresholds [111]. Similarly, Ad-mediated gene transfer of a combination of BDNF and TGF-β provided hair cell and hearing protection against aminoglycoside ototoxicity when injected into the cochlea prior to the ototoxic insult [112]. A regulated AAV gene expression system (tet-on) also successfully improved cochlear function after ototoxic injury by mediating the expression of GDNF [113].

### 6.5 The role of gene transfer in regeneration of cells after SNHL

By far the most outstanding outcome from gene transfer methods is the generation of new hair cells after SNHL in mammals. In amphibians and birds, damage to the cochlear and vestibular sensory epithelia initiates spontaneous cell division and differentiation of daughter cells into new hair cells. This phenomenon does not occur naturally in mammals.

Hair cells and supporting cells in the mammalian organ of Corti originate from a common progenitor; therefore, expression of particular genes may induce supporting cells to change their phenotype to hair cells.

Hair cells are absent in mice that are null for the *Atoh1* gene, the mouse equivalent of the *Drosophila atonal* proneural gene also known on *Math1*, demonstrating the requirement of expression of this gene for the differentiation of the progenitor cell into hair cells [114]. In proof of this, over-expression of *Atoh1* in supporting cells of the organ of Corti *in vitro* resulted in the differentiation of supporting cells into extra hair cells [115]. To determine whether this could be achieved *in vivo* in adult animals, the *Atoh1* gene was injected into normal hearing GP cochleae via Ad. Supporting cells of the organ of Corti were induced to become hair cell-like and remarkably, SGN fibres grew towards the new ectopic hair cells [98]. Detailed analysis of newly generated hair cells from *Atoh1* expression *in utero* in mice demonstrated that the hair cells were morphologically and functionally near-normal [116]. Indeed, four days after induction of SNHL in GPs, expression of the same gene in damaged organs of Corti improved hearing thresholds as a result of new hair cell formation [117]. In addition, after destruction of vestibular hair cells by aminoglycosides in mice, *Atoh1* gene transfer regenerated vestibular hair cells and restored balance to affected mice [118]. A significant impediment to the success of gene transfer to the cochlear epithelium is the sensitivity of supporting cells to SNHL. Supporting cells are severely disrupted 3 – 4 days after aminoglycoside deafening in GPs, with the complete collapse of the organ of Corti after approximately 5 days [119]. Collapse of the organ of Corti also occurs in other species, including humans, albeit with different timelines [5,14,120,121]. Expression of *Atoh1* in the flattened epithelium that remains after complete organ of Corti collapse could not induce the formation of new hair cells [122].

Gene transfer has the potential to protect and regenerate sensory and neural cells in the cochlea after SNHL but the extent of damage and degeneration is a limiting factor to the success of this drug delivery system. Cell-based therapies offer a similar long-term drug delivery solution and may have the ability to replace lost cells when extensive damage has already occurred.

## 7. Cell-based therapy

A significant body of research in non-auditory fields focuses on cell-based therapy for tissue protection, regeneration and replacement. In the auditory system cellular therapy is in its infancy, with researchers predominantly aiming to generate new SGNs or hair cells using stem cells [95,123-128] (reviews [129-131]). Less common but of equal relevance is the use of cell-based therapy for neural protection via the use of cells that secrete neuroprotective proteins such as neurotrophins [70,131].

### 7.1 Cell-based therapy for preserving SGNs

The rationale for neurotrophin delivery via cell-based therapy parallels that for gene therapy, in that it offers sustainable drug delivery from a single application. Rather than genetically altering cells within the cochlea, this paradigm utilizes exogenous cells which secrete neurotrophins naturally or via genetic modification prior to transplantation. Neuroprotective cells transplanted into the cochlea could attenuate SGN degeneration associated with SNHL and therefore be used in conjunction with the cochlear implant to improve hearing outcomes for patients suffering profound SNHL (**Figure 4**).

#### *7.1.1 Cell sources*

Cell transplants may be from autogenic, allogeneic or xenogenic sources. Autologous cell therapies, using the patients' own cells, are a clinically attractive strategy avoiding the potentially serious complications of immune rejection or the need for continuous immunosuppressive therapy. Despite this option being theoretically ideal, the costs, expertise and time involved in creating personalized transplants limits their applicability. Allogeneic transplantation reduces these difficulties because transplants are created from a donor of the same species as the recipient, potentially allowing for an 'off-the-shelf' population of cells that are screened and ready for use [133,134]. However, not all cells can be readily harvested from humans without morbidity or in the required quantities. Xenotransplantation, transplanting cells from one species to another, is being pursued vigorously to solve the shortage of allogeneic donor organs [135]. The major hurdles to the clinical application of xenotransplantation are the severe complications of immune rejection and subsequent immunosuppression and the potential for disease transmission.

#### *7.1.2 Cell types*

For the purpose of protecting SGNs, transplanted cells should naturally secrete neurotrophins or be genetically modified to secrete neurotrophins. In addition to their role of myelinating peripheral nerves, Schwann cells secrete BDNF, NT3, neurotrophin 4/5, NGF, CNTF and leukaemia inhibitory factor [136-138]. The regenerative capacity of transplanted Schwann cells has been extensively described in models of peripheral nerve [133,139-143] and spinal cord injury [144-146]. In various animal models of these injuries, Schwann cells have been observed to promote axonal sprouting, regeneration and long distance regrowth of neurons [147,148] and functional recovery [149-151]. The neurotrophin expression profile of Schwann cells and the endogenous and exogenous ability of Schwann cells to promote neural repair suggest that Schwann cell transplantation will protect SGNs following SNHL. Experimentally, there are preliminary reports from *in vitro* SGN culture experiments that Schwann cells contribute to SGN survival after removal of neurotrophic support by hair cells [152,153].

Cells modified to produce neurotrophins by *ex vivo* gene transfer ameliorate some of the issues associated with

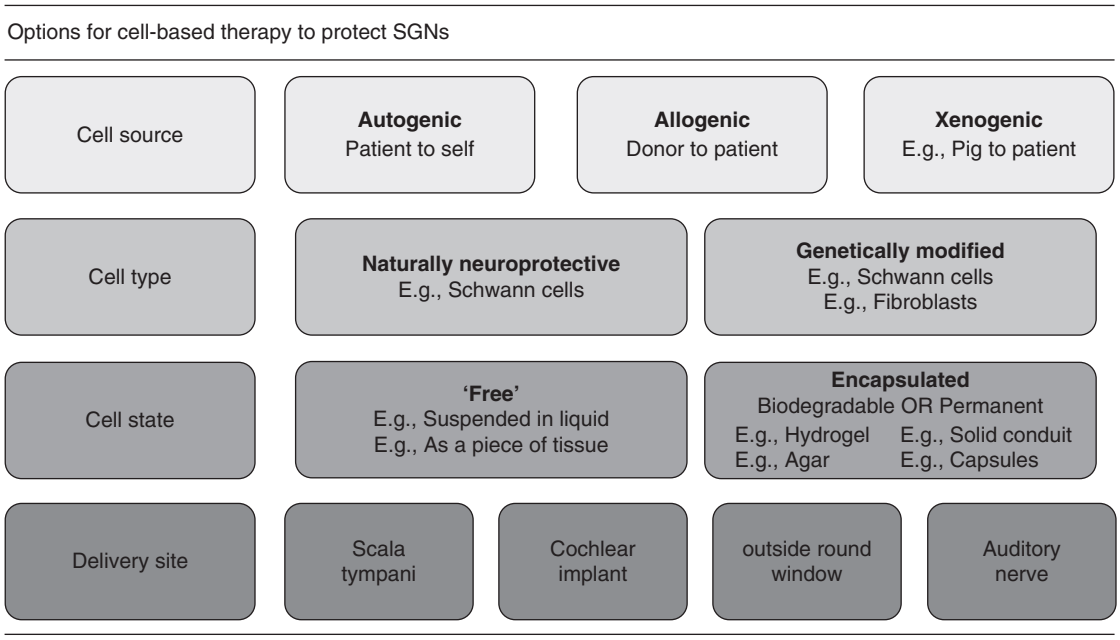


**Figure 4. Flow chart for cell-based therapies for SGN protection in the cochlea after SNHL.** Cells that secrete neuroprotective compounds can be used in the cochlea for preserving SGNs after SNHL. Since the cells do not necessarily need to integrate into the tissue to function, there are a range of cell delivery sites and cell states that may be used. Of significance is the encapsulation of the cell to simultaneously protect the cells from the immune system while still allowing the passage of the therapeutic agent out of the capsule and nutrients into the capsule.



targeting endogenous cells with a viral vector and can be transplanted to the appropriate site in the cochlea. Rejali and colleagues [70] recently demonstrated that an implanted electrode coated with autologous fibroblasts modified to overexpress BDNF reduced SGN degeneration in GPs following SNHL. Similarly, after boosting the secretion of neurotrophins from Schwann cells by genetically modifying the cells with BDNF or NT3, improved SGN survival in culture was observed [132]. The ability of these modified cells to maintain a sustained release of neurotrophins in the cochlear environment long-term is yet to be elucidated.

#### *7.1.3 Cell delivery strategies*

Most studies to date have delivered unencapsulated 'free' cells, suspended in sterile liquid to the cochlea. Delivering free cells into the fluid compartments of the inner ear (for example the scala tympani or vestibular system) allows widespread dispersal and migration. It also allows the exogenous cells to respond to endogenous cues and integrate into the host tissue. For cell replacement therapies, cell migration to the correct site and the resulting cell–cell interactions may be crucial to the success of the strategy. In contrast, as discovered from the introduction of viral vectors into the cochlea, this technique allows the passage of cells from the cochlea through the cochlear aqueduct to the cerebrospinal fluid and even the contralateral cochlea [154]. However, for neurotrophic supply only the molecular products of the cells are required by the target cells, leading to the possibility of encapsulated cell transplantation.

Encapsulating cells in a matrix can prevent the spread of transplanted cells, targeting the cells and their secreted products to a designated region. The BDNF-secreting fibroblasts that were delivered to the cochlea via the cochlear implant were suspended in agar [70]. However, substances such as agar are biodegradable and cannot contain cells permanently. More stable options include synthetic devices such as the Theracyte [155] or alginate capsules, which have a dual outer wall and are proven to hold cells in the human body for over 9 years [156]. Importantly these permanent cell delivery options have been developed to overcome immunological issues without the use of immunosuppressants. They allow the passage of nutrients and cell products such as neurotrophins across their barriers but not antibodies, essentially hiding the cells from the host immune system. This approach enables transplantation of cells from any origin, including xenogenic.

### 7.2 Cell-based therapy for hair cell and SGN replacement

Stem cells are capable of self renewal and differentiation into specialised cell types under specific culture conditions, creating sources of new cells for cell replacement therapy. Cells that express markers for hair cells such as Math1 and myosin 7a have been formed from a subpopulation of embryonic stem cells using growth factors such as epidermal growth factor,

insulin-like growth factor 1 and basic FGF [157]. Likewise, bone marrow mesenchymal stem cells acquired hair cell characteristics, including hair cell bundles, following overexpression of *Math1* (also known as *Atoh1*), creating a potential source of autogenic stem cells for hair cell replacement in humans [158]. For generation of SGNs, mouse embryonic stem cells exhibited characteristics of SGNs when co-cultured with organ of Corti explants from young rats [159], while neural progenitor cells formed similar connections and synapses to SGNs when grafted into the auditory nerve in the cochlea [160,161].

A number of approaches have been used to introduce stem cells into the cochlea for the replacement of hair cells. Mouse embryonic stem cells directed towards the neuroectoderm lineage and expressing the hair cell marker myosin 7a were injected into the scala tympani and scala media fluid-filled spaces. The cells were reported to survive in the cochlear environment for 9 weeks, but there was no evidence of cells integrating into the tissue [123]. In contrast, inner ear progenitor cells with hair cell properties grafted into the otic vesicle in developing chick embryos integrated into the sensory epithelia, particularly in areas of damage [157]. Similarly, relatively undifferentiated clonal neural stem cells injected into scala tympani of the mouse and GP cochlea after noise deafening integrated into tissues such as Rosenthal's canal and organ of Corti. Expression of myosin 7a in cells that integrated into the organ of Corti indicated that the new microenvironment of the cells influenced their differentiation [162].

Significant advances have been made in the area of neuronal replacement in the cochlea. Neural progenitors derived from mouse or human stem cells were co-cultured with sensory epithelia and shown to grow processes towards the epithelia, synapsing with hair cells in some cases [160,163]. When grafted into the nerve trunk of denervated gerbil cochleae, such cells showed integration into the tissue and synapse formation with hair cells [160,161].

Outcomes from transplanting stem cell-derived cell populations into the cochlea appear to depend on the level of differentiation of the cell before transplantation and the region of the graft. However, care must be taken with stem cells that are too undifferentiated as they may form teratocarcinomas [164]. On the other hand, stem cells that are too differentiated may not be able to form appropriate connections or integrate correctly, as they no longer express the early markers or respond to guidance cues required for cell–cell interactions. With advancing research into stem cell therapy that overcomes the safety issues associated with these cells, it is possible that hair cells and SGNs may one day be replaced via a single injection or grafting of stem cells into the cochlea after SNHL.

## 8. Conclusion

The impact of hearing loss on individuals, families and society is often underestimated. However, the degenerative processes associated with SNHL are now well understood and many experimental therapies are being developed that halt or reverse the degenerative processes for restoring hearing or improving outcomes with cochlear implantation. A promising, simple and non-traumatic drug delivery system (dexamethasone placed on the round window membrane via a polymer prior to surgery) led to the preservation of residual hearing after cochlear implantation. This technique would be applicable for any inner ear surgery or other anticipated activities that risk hearing loss (e.g., cisplatin-based chemotherapy). However, there are many cases in which hearing loss cannot be predicted or where extensive degeneration of hair cells and SGNs has already occurred. Since SGN degeneration is progressive over time, preservation of the remaining cells is critical. Neurotrophins are known to protect SGNs after loss of hair cells, and many drug delivery systems are being investigated for safe, short–medium-term drug release combined with chronic electrical stimulation from a cochlear implant. A novel drug delivery biomaterial that meets these criteria is the conducting polymer known as Ppy, which stores and elutes neurotrophins in a controlled manner and conducts the electrical current from a cochlear implant required for auditory perception and the continued maintenance of SGNs. Gene- or cell-based therapies promise long-term drug delivery with the additional benefits of cell specificity, cell regeneration or cell replacement. With more development, one or more of the above-mentioned drug delivery systems may reverse the degenerative aspects of SNHL to improve electro-acoustic hearing or even restore acoustic hearing to normal.

## 9. Expert opinion

Improvements to speech recognition outcomes from cochlear implantation have plateaued in recent years with performance variable across the patient population. Future improvements in speech recognition and sound quality will come from advances in electrode design, combined with biological therapies that prevent degeneration of the cochlear nerve and promote functional regeneration of dendrites.

It is commonly assumed that there is a positive correlation between cochlear implant performance and SGN survival. That is, implant recipients with significantly less SGNs would be expected to perform relatively poorly in speech comprehension tasks. However, there is no direct evidence that the number of surviving SGNs correlates with word recognition or improved speech perception in implant recipients [165]. In fact, a negative correlation between SGN survival and speech comprehension has been reported [166]. These findings suggest that we have reached the limit in sound fidelity that is achievable with contemporary cochlear implant designs. The conductive nature of the cochlear fluids and the proximity of the stimulating electrodes to the neural tissue limit the number of independent sites of excitation due to current spread. Therefore, improvements in sound quality cannot be achieved by simply increasing

the number of stimulating electrodes, but will come from strategies that combine biological therapies, electrode design and current steering techniques to achieve more precise and spatially restricted stimulation. It is under these conditions that greater numbers of SGNs with functional peripheral dendrites would contribute to improved hearing outcomes for implant recipients.

The current trend towards combining cochlear implantation with residual acoustic hearing creates the need to protect the hair cells and SGNs that convey this information. Hearing protection from ototoxic drugs or surgical insult can be achieved following a single dose of protective agents to the inner ear using relatively simple techniques such as application of the agent to the round window membrane. The delivery of protective agents is more time critical for hair cells compared to SGNs due to their greater metabolic vulnerability, the majority of which are lost within hours after a cochlear insult. Therefore, protective agents for cochlear hair cells must be applied before or very shortly after injury. In contrast, drug delivery for SGN preservation can be effective following a longer delay between the onset of SNHL and intervention. However, neurotrophic support must be continuous; hence more complex methods of delivering therapeutic agents are required for long-term SGN preservation. Drug-eluting conducting electrode coatings can provide short- to medium-term neurotrophic support during and after the implantation period, when SGNs can be particularly vulnerable, but would preferably biodegrade after their useful life is over. Long-term neurotrophin delivery for SGN preservation is more achievable via gene or cell-based technologies.

Acceptance of gene therapy for drug delivery system to the inner ear will depend on the outcomes from research into other chronic degenerative diseases. AAV-mediated gene transfer of the glutamic acid decarboxylase gene is already undergoing clinical trials for Parkinson's disease, with promising results in terms of the safety of the procedure as well as improved motor function [167]. As clinical trials continue to demonstrate the safety and effectiveness of such technologies, parallel applications in areas such as preservation of hair cells and SGNs after hearing loss will benefit. Like gene therapy, the field of cell-based therapies is largely driven by other degenerative diseases, principally diabetes. Hence rapid advances will be made in this field for long-term drug delivery. Insulin-producing cells within protective capsules have been implanted into diabetes patients and have survived for over nine years, still secreting insulin. Translating this research to the cochlea using neurotrophin-secreting cells, whether encapsulated or free within cochlear fluids, are known to maintain the health and survival of SGNs in the absence of hair cells, and will provide one solution for the long-term preservation of SGNs.

The SGN survival response to neurotrophins delivered to the cochlea has proven to be robust but the dendritic resprouting is sporadic, uncontrolled and likely to lead to increased variability in cochlear implant performance. However, evidence from gene- and stem cell-mediated hair cell regeneration studies demonstrated that dendrites will grow towards new targets if given the appropriate guidance cues, raising the possibility that it will become possible to control dendritic regrowth. Future research should focus on a combination of therapies such as hair cell replacement/regeneration and neurotrophin delivery to stimulate robust survival and regrowth of SGN dendrites, leading to reinnervation of the cochlea after SNHL and restoration of hearing.

Gene therapy convincingly generated new hair cells from supporting cells but requires surviving supporting cells in the organ of Corti after SNHL. Given the vulnerability of these cells to agents that induce SNHL, it appears that this will not become a common method for replacing hair cells. Cell-based therapies, in contrast, do not depend on cell preservation in the cochlea to be effective. Hair cell and SGN replacement by stem cell therapy shows great promise for regenerating the auditory system. However, it remains to be seen whether stem cells will correctly integrate into the auditory pathway and assume normal function without the significant risk of cancer formation.

In summary, we believe the near future will see drugs such as dexamethasone and neurotrophins being used in the clinic for protecting hair cells and SGNs during and after surgical procedures using minimally invasive short-term drug delivery techniques. The medium-term future will boast the use of smart, slow-release materials and devices that provide protective drugs to the inner ear in combination with advances in cochlear implant design that will deliver electrical current more effectively for improved speech perception. Finally, with continued research into areas of stem cells and gene therapy, restoring hearing by regeneration of hair cells and SGNs in the cochlea will become a clinical reality.

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## Declaration of interest

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