

New strategies for the restoration of hearing loss: challenges and opportunities

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Introduction: For most types of hearing impairments, a definitive therapy would rest on the ability to restore hair cells and the spiral ganglion neurons. The only established technique to treat deafness is based on the functional replacement of hair cells with a cochlear implant, but this still has important limitations.

Sources of data: A systematic revision of the relevant literature is presented.

Areas of agreement: New curative strategies, ranging from stem cells to gene and molecular therapy, are under development.

Areas of controversy: Although still experimental, they have delivered some initial promissory results that allow us to look at them with cautious optimism.

Growing points: The isolation of human auditory cells, the generation of protocols to control their differentiation into sensory lineages, their promising application *in vivo* and the identification of key genes to target molecularly offer an exciting landscape.

Areas timely for developing research: In this chapter, I discuss the latest advances in the field and how they are being translated into a clinical application.

Keywords: hair cells/spiral ganglion neurons/stem cells/gene therapy/regeneration

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Introduction

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The sense of hearing is primordial for our daily interaction with the surrounding environment. Hearing loss carries a substantial emotional, social and economical toll. When is of early onset, it affects the development of speech and language, having implications for social integration. The development of a hearing impairment later in life has a huge impact in our working environment, the way we interact with friends and family and could lead to people feeling withdrawn and ostracized.

In 2004, it was estimated that 278 million people around the globe had moderate to profound hearing loss in both ears (www.who.int/mediacentre/factsheets/fs300/en/index.html). These numbers are likely to rise during the next decades due to the increased noise pollution and the prolonged life span of the population, because of the close relationship of deafness with ageing.

Besides noise and ageing, hearing impairment is also caused by genetic, inherited factors and the prescription of ototoxic drugs.¹ The pathological changes underpinning hearing loss are concentrated mainly to the inner ear, which is the primary centre for hearing. The process of sound perception begins at the *cochlea*, a small organ situated within the temporal bone, which converts the mechanical sound wave into an electrical, nerve-transmitted signal. The cochlea contains two major types of sensory receptor cells, the inner hair cells (IHCs) and the outer hair cells (OHCs). The IHCs are the primary transducers, translating the displacement of their apical hair bundles, induced by the sound wave, into a depolarizing signal. The OHCs, on the other hand, receive efferent stimulus from higher control centres to modulate the auditory signal and contribute to magnify the sensitivity of the system.² The IHC signals are conveyed to higher auditory nuclei in the brainstem via the spiral ganglion neurons (SGNs), the primary order neuron of the auditory pathway. There are several nuclei in the brainstem responsible for adjusting and tuning the signal from the cochlea before sending fibres for final interpretation of sound to the auditory cortex. The loss of sensory cells in the cochlea accounts for the majority of hearing deficits (90%), and is classified as sensorineural hearing loss (SNHL). Such deafness can be caused by primary degeneration of the SGNs, in what is known as auditory neuropathy spectrum disorder, or by the primary loss of hair cells.^{3,4} Secondary degeneration of neurons commonly follows HC loss and cell death occurs due to lack of trophic support.⁵ However, in humans, this process is highly variable and depends on several factors.^{6,7}

Lack of regenerative response in the adult mammalian cochlea

While non-mammalian species can repair and heal their damaged sensory epithelia, the mammalian cochlea does not have the potential to regenerate neither the hair cells nor the sensory neurons.⁸ In avians and lower vertebrates, supporting cells can be triggered by the signal of dying hair cells to replace them by either proliferating or transdifferentiating modes.⁹ Just like in birds, the mammalian supporting cell shares a common progenitor with hair cells during development.¹⁰ However, supporting cells of the mammalian organ of Corti fail to show any

regenerative response to HC loss, via either direct transdifferentiation or mitosis.¹¹ The vestibular organ (including the utricle, the saccule and the cristae ampullaris) is also a mechanosensory structure located in the inner ear that conveys information on position and gravitational acceleration. A mild regeneration of hair cells has been observed in the vestibular sensory epithelia in guinea pigs following ototoxic drug treatment at different time-points.¹² The mammalian vestibular organ, however, has a much simpler cellular architecture than the organ of Corti, more similar to the ears of birds and reptiles. A more recent study on the murine utricle has confirmed that vestibular hair cells can spontaneously regenerate after exposure to the ototoxic drug gentamycin. Large number of immature hair cells could be seen as early as 2 weeks after the lesion. However, neither the regenerated cell numbers nor their appearance were normal.¹³ Burns *et al.*¹⁴ have used an inducible *Cre* allele to drive expression of diphtheria toxin to kill specifically the hair cells. When induced in the utricle of newborn mice, death of hair cells triggers significant mitotic replacement of hair cells *in vivo*. However, when induced in P5 mice, toxin expression caused hair cell loss but failed to evoke a mitotic response. Populations of stem cells can be isolated from the adult mouse utricle,¹⁵ but they virtually disappear from the mouse cochlea after the third week of age.¹⁶ Efforts to characterize these cells in the early post-natal cochlea have identified a population of Lgr5+ supporting cells^{17,18} that are competent to differentiate into hair cells. Lgr5 is a marker of adult stem cells in the intestine, and is expressed by cells in the greater epithelial ridge, inner border cells, inner pillar cells and the third row of Deiter's cells at birth. Although the early post-natal Lgr5+ cells are competent to produce hair cells, this property appears to be lost with age, even when Lgr5 is retained by inner pillar cells and the third row of Deiter's at least until P60.¹⁸

In contrast to cochlear HC progenitor cells, there is some evidence to suggest the presence of neural progenitor cells in the adult auditory nerve. Although Oshima *et al.*¹⁶ failed to isolate stem cells/progenitors from the spiral ganglion of adult mice, Rask-Andersen *et al.*¹⁹ isolated nestin-positive neural progenitor that also expressed TrkB and TrkC from adult human and guinea pig spiral ganglion tissues. Regeneration of SGN cannot be observed after degeneration; therefore, damage to neurons can lead to permanent deafness.²⁰ Even when the cell body and central axon survive, deafness can still occur due to degeneration of peripheral processes.⁶ SGN degeneration has been described in a variety of pathologies. Exposure to sound pressure levels that do not harm HC and cause HC loss can still insult the SGN and trigger neuronal degeneration.²¹ In humans, the regrowth of SGNs does not appear to be clinically significant.⁶ In summary, although there are

indications that some reparative potential is still present in the adult spiral ganglion, recovery does not seem to take place at any substantial rate after damage to HCs or neurons in the adult mammalian cochlea.

The lack of capability of the mammalian cochlea for self-repair makes the deafness ensuing from damage, permanent. Although several promising lines of research are at the moment under exploration, we do not yet have a definitive strategy for auditory regeneration. The only therapy currently available is the use of hearing aids and cochlear implants, prosthetic devices that although highly effective do not fully replace the complexity of the biological organ. Below I will summarize some of the current strategies being pursued to repair and restore the damaged cochlear organ (Fig. 1).

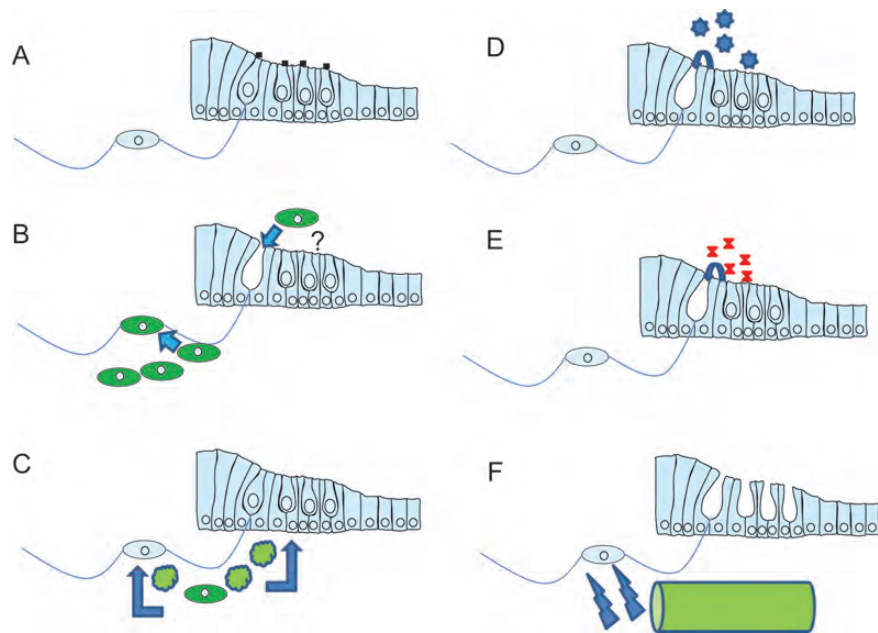


Fig. 1 Potential strategies for sensory cell restoration in the cochlea. (A) Simplified schematic of a normal mouse organ of Corti around the day of birth, displaying a single row of IHCs, three rows of OHCs and an afferent SGN innervating the IHCs. (B) Damaged cells could be replaced by stem cells prepared *ex vivo*. Recent evidence shows that functional restoration of SGN may be possible, while replacement of hair cells still needs to be established. (C) Progression of damage to the sensory cells could be halted by administering growth factors/neurotrophins; these could also protect cells before a trauma (like administration of ototoxic drugs). Exogenous cells modified to produce steady levels of neurotrophins could be used for long-term delivery. (D) Alternatively, manipulation of critical gene using viral vectors could lead to differentiation of supporting cells into hair cells. (E) Along similar lines, critical signalling pathways that control hair cell differentiation could be stimulated by small molecules. (F) Missing sensory cells can be replaced by an electronic device. In this example, hair cells function is restored by a cochlear implanted electrode that stimulates the SGNs.

Potential regenerative strategies

Cell replacement with stem cells

Given the lack of endogenous regeneration and the limited therapeutic range available, the potential to develop a treatment based on the delivery of exogenous cells offers new hopes. Cell-based approaches have been proposed directed to the replacement or restoration of damaged HCs and/or SGNs. Stem cells are excellent candidates for biological implantation as they have the potential to proliferate and differentiate, both required features for regeneration. The optimization of a cell transplantation strategy is a phenomenal task, since there are huge variables to consider in each experimental paradigm. The range of different stem cells and cell lines of potential use, the state of the host tissue and the routes for delivery are all issues that could affect the efficiency of transplantation. These different variables and experimental systems have been reviewed before;²² therefore, I will concentrate on the following paragraphs on the main results obtained using the real putative therapeutic agents, i.e. stem cells of human origin.

Foetal auditory stem cells: a model for cochlear stem cell biology in humans

Despite the advances obtained in rodents, until recently hearing research has suffered from the lack of a suitable model to study stem cell biology of the auditory organ in humans. This started to change when a population of stem cells was identified in the human foetal cochlea,²³ which were later isolated and grown in culture. An homogenous population expressing key stem cell markers such as NESTIN, SOX2, OCT4 and REX1 was selectively expanded by culturing dissociated cells from sensory epithelia from 9 to 11 weeks old foetuses in a serum-free media supplemented with EGF, IGF1 and bFGF.²⁴

Several stem cell lines were established that retained expression of these markers and remained proliferative for several months. When treated with defined culture conditions, they displayed the characteristic bipolar morphology of SGNs and expressed the neuronal markers *NEUROGEN1*, *BRN3A*, b-TUBULIN III and NEUROFILAMENT 200. Moreover, 5–7 days after inducing differentiation, bipolar cells displayed potassium delayed rectifiers and voltage-gated sodium currents. On the other hand, when culture in the presence of Retinoic Acid and EGF, hair cell-like phenotypes were induced as measured by the expression of *ATOH1* and *BRN3C* as well as MYOSIN VIIA and PARVALBUMIN. Furthermore, these cells showed a rearrangement of the actin cytoskeleton, resembling the cuticular plate, and expressed

the inward rectifier K^+ current (I_{K1}), a small outward I_K and a sustained inward Ca^{2+} current.

Human embryonic stem cells as a source for otic progenitors

Efforts to produce sensory neurons from human embryonic stem cells (hESCs) are also producing encouraging results. hESCs were induced to form embryoid bodies and latter transferred to differentiation media in the presence of NT-3, BDNF, FGF or bone morphogenetic protein 4. The hES-derived neuroprogenitor cells projected fibres to denervated *ex vivo* sensory epithelia and expressed synaptic markers. When the neuroprogenitor cells were transplanted in the cochlear nerve trunk of deafened animal, they engrafted and sent out processes which grew towards the auditory sensory epithelium.²⁵ Independently of this work, functional and specific auditory sensory neurons have been produced from hESCs using a step-wise protocol that generates otic progenitors.²⁶ Two types of progenitors were obtained, otic neuroprogenitors (hONPs) and otic epithelial progenitors (hOEPs). hOEP can produce hair cell-like cells and SGN when manipulated *in vitro* like the human foetal auditory stem cells (hFASCs), while hONPs are committed to the neuronal lineage. These cells survived, differentiated and grew neurite projections when transplanted into deafened gerbil cochleae but, more importantly, showed robust evidence of functional recovery.²⁷

Other types of human stem cells

Besides the hFASCs and hESC-derived otic stem cells, other populations have been explored for their potential application to the ear. Revoltella *et al.*²⁸ transplanted CD133+ human haematopoietic stem cells to deafened mice. Treated animals showed promising signs of histological repair, although there was no indication of cell integration or functional recovery. It is possible that these cells were acting in a paracrine fashion, helping the healing process. Olfactory stem cells have been used on A/J mice, a model of early onset, progressive hearing loss.²⁹ When administered just before the onset of damage, they moderately prevented degeneration. The transplanted cells were mesenchymal in nature and did not become incorporated into the cochlea, suggesting that the effect could also be indirect, perhaps mediated by secreted molecules. Nevertheless, it has been shown that human mesenchymal stem cells have the potential, at least *in vitro*, to differentiate into otic sensory cell lineages.^{30,31}

Cell preservation by growth factors

There have been numerous attempts to protect and prevent HCs and SGNs from degeneration triggered by drugs, noise or age by treatment with neurotrophic factors (NTFs) or exogenous reagents. The major exogenous compounds that have been applied to the auditory system are neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF).

Neurotrophin secretion is reciprocal among HCs and SGNs.³² The production of NT-3 is crucial to the survival of type 1 SGNs during development of IHCs innervation, whereas BDNF is required for SGN type 2 survival.³³ There is also an expression code placed along the longitudinal axis of the cochlea during development. Early in development, BDNF is secreted at the apex, while NT3 is produced at the basal and middle turn. Towards the end of the embryonic development, both domains of expression have merged; however, BDNF retains its higher expression at the apex, while NT3 is primarily produced at the base.³⁴ This topical dependency would seem to be only relevant during development since, as highlighted below, application of BDNF appears to have a protective effect on the basal SGNs.

Degeneration of adult SGNs can be prevented by infusion of NT-3.^{35,36} These efforts have shown the benefit of NTFs supplementation in reducing the degeneration of SGNs secondary to HC loss, suggesting possible clinical improving for the cochlear implantation. It has been shown that infusion of a combination of BDNF and ciliary neurotrophic factor into the cochlea can enhance the survival of SGNs and also restore the evoked auditory brainstem response (eABRs) after chemically inducing deafness and mimicking the cochlear implantation by introducing a platinum–iridium electrode to deliver electrical stimulation.³⁷ Recently, Agterberg *et al.*³⁸ have shown a significantly improvement of eABR thresholds after BDNF treatment via osmotic pump 2 weeks after cessation of treatment in the HCs degeneration model induced by kanamycin. Although NTFs increased SGN survival after HC degeneration producing a functional improvement, they have not yet been tested in a real clinical condition and their application remains unproven. However, once their safety for human application is established, they could be of clinical use as supportive treatment during cochlear implantation.

Delivery of NTFs is a key issue, and most of the reported experiments employed a pump for sustained intracochlear administration. Havenith *et al.*³⁹ have used gelfoam to apply BDNF onto the round windows of deafened guinea pigs. This local application enhanced the survival of the SGNs in the basal cochlear turn; however, eABR amplitudes were not substantially improved. Although this method of delivery is less

intrusive and has some effect, it is limited when compared with intracochlear administration.

One of the main limitations of any treatment that involves neurotrophins is not only the route of delivery, but also how to sustain administration for a long period of time. Transplanted cells have been proposed as a way to dispense these factors long term. As a safety consideration of this approach, cells can be inserted encapsulated (since do not need to establish any physical connection) in a material that would let diffuse the secreted NTFs. This capsule should facilitate their removal if anything fails to go as planned. Using this concept, encapsulated BDNF-over-expressing Schwann cells have been delivered to deafened guinea pigs, improving SGN survival.⁴⁰ Moreover, encapsulated choroid plexus cells have been delivered into deafened kittens together with cochlear implants. Remarkably, combined electrical stimulation with cell-based NTFs delivery produced the best results as measured by increased cell survival and density of the peripheral processes.⁴¹

Cell restoration by genetic manipulation

Cell survival and regeneration in the inner ear could be achieved by over-expressing and/or down-regulating key genes.

An important point to consider for the development of a gene therapy strategy for deafness is the use of viral vectors that can effectively transduce the appropriated cell type without substantial, long-term complications. A considerable amount of work is being dedicated to develop goods systems, and they have recently been reviewed by Lustig and Akil.⁴² In general, adenovirus and adenovirus-associated vectors (AAV) seem to be very effective for gene delivery into the cochlea. When applied to the adult guinea pig cochlea, replication-deficient adenovirus (using the cytomegalovirus promoter) drove transgene expression to IHCs and pillar cells. AAV transduction tested with several promoters (such as platelet-derived growth factor, neuron-specific enolase and elongation factor 1alpha promoters) directed transgene expression to cochlear blood vessels, nerve fibres and spiral limbus cells, respectively.⁴³ When delivered *in utero* into the otocyst, adenovirus efficiently transduced supporting cells, but elevated ABR thresholds after birth. Bovine adeno-associated virus on the other hand showed good tropism for the supporting cells and neurons, without compromising auditory function.⁴⁴ A recent paper by Akil *et al.*⁴⁵ has opened new hopes for therapeutic gene delivery to repair damaged hair cells. AAV1 particles carrying the VGLUT3 gene reconstituted gene expression in IHCs from null mice. These animals had morphological

improvement of their ribbon synapses and functional recovery of their ABR thresholds.

Neurotrophins

As discussed before, NTFs have a protective effect on SGNs and enhance their survival after hair cell loss. Another way of delivering NTFs into the cochlea is by genetic manipulation. In this manner, it could be possible to circumvent the delivery challenges imposed by the long term, sustained availability required to maintain NTFs efficacy. Adenoviral vectors were used to deliver a GFP reporter together BDNF and NT3 into the scala media of deafened guinea pigs, targeting several cell types efficiently. However, when cochleae were infected after prolonged deafness (8 weeks), the levels of expression of the transgenes were reduced, as was their effect on SGN survival. These findings suggest that although there is potential for the clinical application of this strategy, the window of opportunity is relatively narrow for the therapy to be effective.⁴⁶

Induction of new hair cells by force expression of Atonal homolog 1 (Atoh1)

The transcription factor Atoh1 (formerly known as Math1) is a key regulatory gene high up in the hierarchy responsible to control the hair cell phenotype. Attention focused on this gene when Bermingham *et al.*⁴⁷ showed that its targeted deletion led to the specific loss of hair cells. Its importance was further realized when deafened guinea pigs cochleae were transduced with Atoh1-adenovirus particles. Forced expression of this gene on the damaged organ of Corti induced the differentiation of new hair cells. A peculiar feature of these novel hair cells is that they were contacting the basal membrane, suggesting that they were in fact transdifferentiated from supporting cells. Remarkably, these new hair cells contributed to restoration of the ABR thresholds.⁴⁸ Unfortunately, the generation of hair cells seems to rely on the integrity of the surviving supporting cells. Seven days after the ototoxic injury that kills the hair cells, supporting cells are replaced by a flat epithelium. When Atoh1 expression was forced on these flat cells using the same viral vectors as before, no evidence of hair cell differentiation was obtained.⁴⁹ It would seem that once the wounded epithelium heals and the injury becomes chronic, the flat cells that replace the supporting cells are not longer competent to respond to Atoh1. Therefore, research aimed to address the nature of these cells and the events occurring after damage is fundamental to facilitate this approach.⁵⁰ Generation of new hair cells and improvement of balance function has also been described for the vestibular organ.⁵¹ However, the transgene was delivered 11 days after damage. Remain to be established if the application after

a longer window post-trauma would be more effective than in the cochlea. New, recent studies have explored if the forced expression of Atoh1 in specific supporting cell types and during defined windows of post-natal development can induce new hair cells. Although efficient in generating new, ectopic hair cells in early post-natal days, the competence of the supporting cells is reduced dramatically by post-natal day 14.⁵² In a parallel study, Liu *et al.*⁵³ showed that although pillar and Deiters cells can produce new hair cells upon forced Atoh1 expression, this ability is completely lost by P30, even after the OHCs have been damaged by kanamycin and furosemide. These new data would suggest that activation of Atoh1 alone is not sufficient to drive new hair cell formation in an adult cochlea, and other factors may be necessary.

Down-regulation of cell cycle inhibitors

Cell cycle regulators are key factors in maintaining the post-mitotic state of the supporting cells, and as such are attractive candidates to target in order to stimulate proliferation and repair.

p27^{Kip1}, a cyclin-dependent kinase inhibitor (CDKI) is up-regulated in the cochlear epithelium at the same time that terminal mitosis begins during embryogenesis, and it remains in the supporting cells till adulthood.⁵⁴ Targeted ablation of the p27^{Kip1} gene leads to the production of supernumerary supporting and hair cells, suggesting it to be a critical negative regulator of hair cell production during development.⁵⁵ Evidence has been presented that a perinatal population of supporting cells can down-regulate p27^{Kip1} *in vitro* and re-enter the cell cycle. This ability is severely reduced by the time they reach to P14. However, even at this stage, a small proportion of cochlear supporting cells can trans-differentiate *in vitro* into hair cells-like cells.⁵⁶ Supporting further the idea that the ability of these cells to proliferate is p27^{Kip1}-dependent, cells taken from null animals had an enhanced proliferative capacity in culture when compared with wild-type ones.⁵⁶ In summary, the level of expression of p27^{Kip1} protein remains robust in differentiated cochlear supporting cells *in vivo*, suggesting that p27^{Kip1} imposes strong inhibition on cell mitosis and may prevent them from dividing after damage. The inhibition of supporting cell proliferation seems to be a major factor that blocks the possibility of hair cell regeneration in the mammalian cochlea. It is conceivable then to develop a therapeutic approach that would down-regulate this gene. However, this strategy will have to deal with potential tumorigenesis due to unrestrained cell proliferation.

Similar to p27 Kip1, conditional ablation of Retinoblastoma (Rb) gene leads to supernumerary hair cells and supporting cells in the developing cochlea.⁵⁷⁻⁵⁹ This finding raised great enthusiasm as a probably target; however, it was not clear whether this effect was only present in the

developing progenitors or also obtainable if adult, post-mitotic cells were to be targeted. Rb deletion in post-mitotic hair cells (using an Atoh-Cre inducible system) led to some hair cells re-entering cell division but failing to differentiate and dying, leading to complete deafness.⁶⁰ On the other hand, when Rb was deleted from two post-natal, supporting cell populations (pillar and Deiters' cells using a *Prox1-CreER^{T2}* line), these supporting cells proliferated and survived for about a week but failed to differentiate into hair cells and die probably from lack of trophic support.⁶¹ So, although potentially promising, any strategy involving down-regulation of a cell cycle inhibitor will need to be combined with induction of differentiation, probably by activating Atoh1 signalling.

Manipulation of signalling pathways using small molecules

Relevant signalling pathways could be modulated by using small molecules with good access into the cochlear compartments. For instance, de-differentiation or transdifferentiation of the supporting cells into hair cells could be elicited by this approach. There is some limited evidence of transdifferentiation occurring in mammals during development. Experiments have shown that new hair cells are generated when existing ones are ablated in the mouse organ of Corti prior to E16, but this ability is lost after E16.⁶² Early post-natal rats treated with the ototoxic drug amikacin generated cells that had mixed features between hair and supporting cells. These cells have been interpreted as having attempted direct transdifferentiation but failing to develop complete hair cell characteristics.⁶³ Together, these findings suggest that the ability of auditory SCs to undergo direct transdifferentiation is limited over the developmental process. Compounds that mimic these events early in development could have a potential clinical application.

Equally, expression of the genes described above could be directly manipulated by specific compounds, without the need of using viral vectors. Up-regulation of Atoh1 by exogenous signals could prove very useful. Using neuroblastoma cells and neuroprogenitors, Shi *et al.*⁶⁴ have shown that Wnt canonical signalling (mediated by β -catenin) can trigger a direct up-regulation of Atoh1 by acting on elements on its 3' enhancer.

Replacing cells with electronic devices

Finally, the use of non-biological electronic devices, in the form of the cochlear implant, has made a huge impact in restoring functionality to

the hearing organ. The first attempts for cochlear implantation were performed in the 1950s by Djourno and Eryies, a French surgeon and an engineer who placed a coil of wire in the inner ear of two deaf patients. Although these trials failed after a short time, they kick-started an area of research that was going to deliver substantial advances.⁶⁵ A modern cochlear implant is an electronic device that can be divided into two major parts: the external head piece that includes a microphone and speech processor and is placed on the skin close to the temporal bone area, and the internal cochlear electrode. The external component functions as a transmitter to process sound signals and is connected to the receiving coil which, secured on the temporal bone beneath the skin, is responsible to convert the signals into electric impulses and deliver them through an internal cable to the implanted electrode in the cochlea. An array of up to 22 electrodes wound up through the scala tympani of the cochlea stimulates the SGNs, which in turn sends the information to the brain via the auditory nuclei. The cochlear implant can give a quality of sound discrimination fine enough to understand speech but post-implantation rehabilitation is critical for ensuring the effectiveness of treatment. Modern cochlear implants allow the typical patient to understand more than 90% of words in unfamiliar sentences when presented in quiet listening conditions.⁶⁶

Because many patients were unable to benefit from cochlear implantation due to an auditory nerve dysfunction, this led to the development of the auditory brainstem implant (ABI). The principle of ABI is similar to cochlear implant but bypasses the function of SGNs by stimulating the cochlear nucleus directly via surface-mounted 'button' electrodes.⁶⁷ This requires an electrode implanted directly into the brainstem, making this device more risky and still not widely used. The best performance of ABI is still poor when compared with cochlear implantation.⁶⁷

Some original developments are taken place that could add a new dimension to the implant of the future. For instance, a membrane of a piezoelectric material inserted into the cochlea of a deafened guinea pig, was able to transduce a sound stimulus into an electrical signal that, in turn, induced an auditory brainstem response. Although still at its very early stages, is an example of a novel and exciting new approach that could change the way we design implants in the years to come.

Finally, although the electronics and software control of cochlear implants is under constant improvement, is likely that the next leap in performance quality will come by combining electronics with biology in a true bionic prosthesis. For instance, SGNs originated from stem

cells could be used in combination with electrodes for the new generation of implants.

Conclusions

The hearing field is witnessing a promising era, where new concepts and developments are becoming close to their clinical application. The next decade will likely see some of the lines of research discussed here consolidated and, hopefully, starting to bear fruits in the clinical arena. It is clear from the material presented above that no 'good for all' strategy is possible, and different conditions would have to be targeted with different approaches, and what is probably good for one type of lesion, may not work well in a different context. For instance, cell preservation by soluble factors could be a reasonable prophylactic strategy for people with a potential high risk of sensory cell loss, such as those exposed to loud noise or undergoing treatment with ototoxic drugs. Conversely, those already with sensory cell loss could benefit from cell restoration by cellular or molecular means. Different strategies should not be mutually exclusive, but, on the contrary, it is more likely that initial success would come from a combination of techniques (i.e. stem cells with cochlear implants). Although invariably some lines of research will fail to translate, it is clear we are living a very exciting time.

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