REVIEW ARTICLE

Perspectives for the Treatment of Sensorineural Hearing Loss by Cellular Regeneration of the Inner Ear

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Sensorineural hearing loss; Stem cells; Inner ear regeneration; Spinal ganglion neurons; Gene therapy

Abstract  Sensorineural hearing loss is a caused by the loss of the cochlear hair cells with the consequent deafferentation of spiral ganglion neurons. Humans do not show endogenous cellular regeneration in the inner ear and there is no exogenous therapy that allows the replacement of the damaged hair cells. Currently, treatment is based on the use of hearing aids and cochlear implants that present different outcomes, some difficulties in auditory discrimination and a limited useful life. More advanced technology is hindered by the functional capacity of the remaining spiral ganglion neurons. The latest advances with stem cell therapy and cellular reprogramming have developed several possibilities to induce endogenous regeneration or stem cell transplantation to replace damaged inner ear hair cells and restore hearing function. With further knowledge of the cellular and molecular biology of the inner ear and its embryonic development, it will be possible to use induced stem cells as in vitro models of disease and as replacement cellular therapy. Investigation in this area is focused on generating cellular therapy with clinical use for the treatment of profound sensorineural hearing loss.

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PALABRAS CLAVE
Hipoacusia neurosensorial; Células madre; Regeneración oído interno;

Perspectivas para el tratamiento de la hipoacusia neurosensorial mediante regeneración celular del oído interno

Resumen  La hipoacusia neurosensorial es un problema que se debe principalmente a la pérdida de células ciliadas cochléara, con la consecuente desaferentación de las neuronas del ganglio espiral. En los humanos no existe regeneración celular endógena en el oído interno, ni


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Introduction

The structure of the inner ear, one of the most fascinating in the human body, includes the vestibular system and the cochlea. These participate respectively in balance perception and hearing. The spiral organ of Corti, housed in the membranous labyrinth, inside the cochlear duct, is formed by a great variety of cell types. Its complex architecture constitutes an example of functional specialization achieved during the evolution of mammals (Fig. 1).

Hearing loss affects some 278 million people around the world, according to the World Heath Organisation. Most are cases of sensorineural hearing loss from cochlear hair cell (CHC) alterations, although some are from changes to the associated neurons due to deafferentation from the loss of trophic factors.1,2 Auditory neuropathy, which causes deafness through dysfunction or primary degeneration of the auditory nerve, is less frequent.2,3 Several genetic environmental factors are involved in this process, such as mutations, viral infections, autoimmune disorders, chronic noise exposure, age, and ototoxic drugs; there are also other factors that cause similar degenerative alterations.4,5 This permanent hearing loss stems from the incapacity of the cochlea to regenerate the CHCs (in contrast to the vestibular hair cells, which can be regenerated in a limited number).6

The only way to restore the auditory function of injured CHCs is currently direct stimulation of the auditory nerve by using cochlear implants or hearing aids in moderate–severe hearing loss. Both are greatly limited due to the complex pathogenesis of hearing loss and degree of tissue damage.7 The ideal solution would be preventing CHC loss or regenerating CHCs and damaged spiral ganglion neurons (SGNs) using stem cell (SC), gene or drug therapies.7,8 If CHC loss also affects SGNs, our ideal therapy would have to include substituting both of them.

Hearing loss involves a lack of spontaneous endogenous proliferating capacity. This makes using exogenous cells or activating supporting cells necessary, using gene therapy to induce their differentiation towards in situ hair cells.8,9

Ongoing research in this area is helping sensorineural hearing loss treatment to become a combination of gene therapy, cell therapy and cochlear implant (Fig. 2).

Mechanisms for Cell Regeneration in the Inner Ear

In mammals, the supporting cells of the organ of Corti, which surround the CHCs, are capable of returning to the cell cycle, dividing and substituting the damaged CHCs. The supporting cells, which express Lgr5, are the Deiters cells and inner hair cells, which can differentiate into CHCs. This differentiation has been demonstrated in the newborn mouse, after ototoxic damage, by drug inhibition of the Notch-activated signals.10 There is no functional cell regeneration in adults. However, several studies have found SCs in el inner ear (0.025% of the total cells of the utricle), which transdifferentiate and proliferate in response to CHC loss.1,6,11 When transferred to an appropriate environment (for example, hen otocyst) these cells can differentiate into various cells types, such as ciliated cells.12 Cultivating these cells in vitro can restart the cell cycle; they divide and transdifferentiate into CHCs, identified by myosin expression.6 This population of mammal utricle SCs significantly decreases after birth and disappears from the third week of life.1,4,12

The supporting cells of mammal cochlea share, during development, a common progenitor with bird CHCs. However, even though these Lgr5+ supporting cells are present in adult mammals, they cannot regenerate spontaneously in response to CHC loss.4

The vestibular organs (utricle, saccule and semicircular canals) have simpler cell architecture, similar to the inner ears of birds. These organs display immature hair cells 2 weeks after an induced vestibular lesion.4 The atonal homolog 1 (ATOH1) gene codes the transcription factor Math1, which is necessary for forming the neurons and internal hair cells of the cochlea. Some adult mammal supporting cells maintain SC characteristics. These cells, when treated with a vector overexpressing ATOH1, improve morphology
(increasing the number of cilia in the CHC); they also produce functional recovery of brainstem evoked potentials as compared to untreated ears.\textsuperscript{4,7} Tissue response to \textit{ATOH1} overexpression depends on the hearing loss mechanism, time of treatment after deafness and degree of differentiation of the surviving nonsensory cells.\textsuperscript{12}

Other cells with SC potential that can be found in the adult mouse cochlea are the mesenchymal. These derive from haematopoietic stem cells (HSCs) in the bone marrow. Although these HSC-derived cells differentiate mainly in macrophages and fibrocytes, it is possible that they can differentiate in CHCs and substitute them.\textsuperscript{12}

The SGNs are the primary afferent neurons of the auditory system. They send projections to the CHCs and to the brainstem, under the control of numerous molecular signals during embryonic development.

Unlike CHCs, there is some evidence of neural progenitors in the adult auditory nerve. The expansion and differentiation of endogenous cell sources (whether spontaneously or following CHC stimulation by drugs, gene therapy or paracrine factors) leads to SGN regeneration and subsequent functional recovery.\textsuperscript{4,13} However, despite this regenerative potential that the adult mammal spiral ganglion has, auditory recovery seems not to occur after primary SGN or CHC injury (even when the cell body or central axon survives).\textsuperscript{4,13}

Peripheral fibres enhanced by neurotrophic factors have been shown to grow spontaneously towards CHCs, but only in an \textit{in vitro} newborn mouse model.\textsuperscript{13,14}

**Mechanisms of Spiral Ganglion Neuron Preservation**

There are 3 mechanisms that can guarantee SGN survival, when they are activated shortly after CHC injury:

**Figure 1** Morphological structure of the anterior labyrinth or cochlea, which contains the organ of Corti, placed above the cochlear duct.

**Figure 2** Strategies for inner ear cell regeneration.
neurotrophic factors, electric stimulation and neuronal apoptosis inhibition. If these factors fail or therapy is delayed for several months, SGN loss is greater. Consequently, any alternate therapy would have to include SGN substitution through an exogenous cell transplant.

**Neurotrophins**

Developing neurons depend on neurotrophic factors (NTFs) for their axons and synapses to survive and mature. Lack of NTFs limits their development and prevents synaptic connections with other neurons from being made correctly.²,¹²

Proteins secreted principally by the organ of Corti, the NTFs constitute the first signals that maintain SGN survival after deafferentation.⁴ Table 1 describes the characteristics of the main NTFs in the cochlear ganglion: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NTF-3 and NTF-4. The organ of Corti is the primary source of NT3 and BDNF in the mature cochlea. However, the SGNs also receive NTF from other sources, including the cochlear nuclei, Schwann cells and the SGNs themselves.²,⁴

There are other protein/trrophic factors that also participate in SGN development and maintain their postnatal survival after deafferentation: for example, TGF-β, gliadervised neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF) and fibroblast-derived factors 1 and 2 (FGF-1, FGF-2).³,¹³ All these factors activate different receptors that use separate intracellular signals, promoting survival or inhibiting cell death synergistically. Consequently, many neuroprotection strategies include a combined action by 2 or more factors.²

Infusing a combination of BDNF and CNTF in the cochlea increased SGN survival and re-established auditory evoked potential response in chemically-deaf guinea pig when used together with electric stimulation.⁴ Applying BDNF to guinea pig oval window increased SGN survival in the basal cochlear turn, but did not improve the amplitude of the evoked potentials.⁴ Other studies have shown that infusing BDFN and FGF-1 together in the tympanic duct at 4 days, 3 weeks and 6 weeks after CHC injury increases survival of the SGNs and of the peripheral axons in guinea pig auditory nerve.⁷

Human experiments with NTFs are limited because of the short half-life, low pharmacokinetic properties, proteolytic degradation and the difficulty in crossing the blood–brain barrier when NTFs are administered systemically. However, recent advances in drug administration techniques, such as gene therapy, cell transplants, NTF micro-capsulation and the design of smaller NTF molecules, may allow these factors to be administered continuously.¹⁵ In addition, NTF can be introduced directly into the oval window when a cochlear implant is placed. This reduces the effect of the blood–brain barrier¹⁵ and improves NTF survival and axon density.⁷ However, phase I clinical trials are needed to establish its safety in human beings, so that it can be used as a support treatment along with the cochlear implant.⁴

### Electric Signals Increase the Survival of Spiral Ganglion Neurons

Electric stimulation has several positive in vivo and in vitro effects on transplanted cells, improving SGN survival and SC differentiation to in vitro neuronal phenotype.¹⁶ The electric activity in membrane, depolarisation with subsequent intracellular Ca²⁺ release and activation of pro-survival signalling cascades are required to transmit sensory information towards the central nervous system (CNS) and can be involved in establishing and maintaining afferent cochlear innervation.⁶ This response to electric

<table>
<thead>
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<th>Protein</th>
<th>Length</th>
<th>Receptors</th>
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<td>Nerve growth factor</td>
<td>241 AA</td>
<td>NTRK1, NGFR</td>
<td>Development and maintenance of the sensory and sympathetic nervous system. Control of proliferation, differentiation and survival.</td>
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<td>Brain-derived neurotrophic factor</td>
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<td>Trk, LNGFR B</td>
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<td>Neurotrophin 3</td>
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<td>Differentiation and maintenance of neuronal subpopulations in the central and peripheral nervous systems.</td>
</tr>
<tr>
<td>NT4</td>
<td>Neurotrophin 4</td>
<td>260 AA</td>
<td>TrkB</td>
<td>Differentiation and maintenance of neuronal subpopulations in the central and peripheral nervous systems.</td>
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stimulation is independent and cumulative to that induced by the NTFs, because they use different receptors and intracellular messengers, which is what increases SGN survival. In animal models, short intermittent stimulation is enough to rescue SNGs, if the stimulation begins shortly after deafferentation. However, results in humans are less satisfactory than those of animal models.  

Cell Death Inhibitors That Prevent Cell Degeneration

Inhibiting cell death signals in deafferented SGNs is an alternative to trophic factors for inhibiting auditory nerve degeneration. Identifying the mechanisms of cell death has made it possible to identify new therapeutic targets, such as oxygen-reactive species, c-Jun N-terminal kinase, proteases and proapoptotic members of the Bcl-2 family. The use of these cell death inhibitors presents a potential advantage: protecting the other cells in the cochlea, including the hair cells.

Strategies for Inner Ear Cell Regeneration

The mechanism that determines the quiescence of inner ear SCs is beginning to be understood. If the proliferation and differentiation of supporting cells, which surround the CHCs in adults, could be stimulated, the injured inner ear could be regenerated, acting on the genes responsible for this activation. However, further studies are needed to ascertain the Notch-activating signals in endogenous SCs. Consequently, exogenous cell transplant has great potential for sensorineural hearing loss treatment, especially given the therapeutic limitations of the implantable devices.

There are 2 strategies for recovering hearing in a patient with sensorineural hearing loss using cellular regeneration:

1. Regenerate the organ of Corti by repairing or regenerating the different cell types that form it, which include from sensorineural hair cells up to highly specialised supporting cells.
2. Regenerate the cochlear ganglion of Corti and the cochlear nerve by generating new SGNs.

Manipulating the SCs directly in the inner ear would provide the perfect environment for regenerating new cells. The same principals could also be used with exogenous SCs. In vivo generation of inner ear cells from embryonic stem cells (ESCs), adult inner ear SCs and neural SCs, with good neuron integration in the cochlea, represents an important advance. Nevertheless, it is not enough just to transplant SCs in the cochlea and wait for the cochlear microenvironment to activate the development programme to regenerate the organ of Corti by itself, as it possesses insufficient local regulators in the adult. The SCs have to be first induced towards neural progenitors and then these have to be induced to sensory neurons using molecular signals; finally, NTF is applied as well to facilitate the brain-inner ear connection.

One of the advantages of cell transplants is that the SCs can be isolated, expanded and directed in vitro towards a specific cell type. There are, however, several challenges in the use of SCs in the cochlea:

1. Differentiation of the cells into an appropriate neural phenotype (with specific electrophysiological characteristics).
2. Effective transplant in the cochlea (including avoiding immunological rejection, which seems low in SNCs).
3. Functional integration of the new neurons in endogenous cochlear and brain structures, restoring the auditory circuit through synapses with the CHCs and cochlear nuclei.

The experimental results obtained have been variable, with differentiation of some of the cells into neurons and glia cells (and possibly hair or support cells) and loss of other cells. In practice, the cell colonies are selected by their morphology and are expanded to obtain sufficient cells; finally, colony differentiation is tested using the expression of specific cellular markers through flow cytometry or immunohistochemistry. However, these markers cannot tell if they are really functional cells.

Regeneration of the Organ of Corti

The complex structure of the organ of Corti makes it difficult to regenerate it. Generating various types of cells, including hair, sensorineural and supporting cells, would be necessary. Another problem is there is only limited capability for cellular migration to the injury site. This is due to the scarcity of chemotactic factors and to the fact that supporting cells immediately occupy the place of the injured CHCs to prevent the endolymphatic space from communicating with the perilymphatic, taking up the space for the transplanted cells. The paracrine environment of the cochlea is essential for transplant survival (in the first 3 days post-injury there is greater survival than from the 7th day onward).

The endolymph also contains high levels of K⁺, which is toxic for the cells transplanted into this compartment. The basilar membrane (rich in collagen) is also an obstacle for cellular migration towards the tympanic duct. However, the greatest difficulty in obtaining functional CHCs is tonotopy. With structural CHC specialisation that reaches the molecular level in the number and activity of potassium channels, this specialisation is vital for the perception of sound frequencies. The CHCs are not homogeneous throughout the cochlea, and this basal-apical and inner-outer distribution causes such specialised hearing. Likewise, to recover function, the new transplanted CHCs must attract and make synapses with the appropriate SGNs to maintain tonotopicity. Furthermore, an inappropriate number of cells or cells in inappropriate condition would case important distortions in sound and would have very serious effects on hearing.

Some studies suggest function recovery would be possible without having to follow the embryonic development programme of the organ of Corti. Any cell capable of efficiently transducing a mechanical stimulus and activating an afferent neuron could function as an effective substitute for an injured cochlea, and generating the entire cochlear structure would be unnecessary. By generating a continuous sheet of generic hair cells of bird auditory papilla type, the trophic stimulus needed for auditory neurons would be obtained, improving the result of cochlear implants.
Cochlear Ganglion Regeneration

Applying SCs offers greater therapeutic potential, such as its use in SGN regeneration.\textsuperscript{12,15} Injury-derived neural deafferentation and CHC loss cause the auditory nerve to degenerate and SGN cell bodies to be lost through NTF deprivation.\textsuperscript{15} The cochlear implant re-establishes hearing by overtaking the damaged sensory epithelium and presenting the codified electric signals directly to the SGNs; implant benefits depend on the density and excitability surviving SGNs.\textsuperscript{15} Advances in implant technology now include higher stimulation rates, more complex stimulation strategies, restricted voltage fields and closer and closer placement of electrodes to the auditory nerve. There will be demands for more and more, to obtain an optimal result. While 10% of the SGNs are enough for initial success, SGN degeneration becomes a medium-range limiting factor for obtaining the desired results in more advanced cochlear implants.\textsuperscript{2} It is to be expected that these continue working during several decades from their activation, and that their appropriate functioning will depend on preserving a sufficient number of SGNs for vocal intelligibility.

It has been demonstrated that SC-based neurons have been generated that extend axons, in a primitive manner, towards the organ of Corti and innervate \textit{in vitro} and \textit{in vivo} CHCs.\textsuperscript{12,16} However, differentiation towards targeted lines, such as SGNs, is limited and there is no evidence of formation of a functional synaptic circuit or auditory recovery.\textsuperscript{16} This is probably the result of a lack of a specific neuronal marker that characterises the SGNs, and of the difficulty in generating a homogenous SGN population from an SC that produces almost any kind of cell.\textsuperscript{16}

The SCs have to be capable of differentiating towards neural progenitors and then SGNs. These should be mostly type 1 SGNs, be derived from humans, have specific electrophysiological properties and integrate themselves functionally with the endogenous inner ear and brain structures, avoiding immune rejection. The phenotypes of the type 1 and 2 SGNs are presented in Table 2.

To condition SC differentiation towards SGNs, the same molecular principals of embryonic development of the otic placode apply. The less the transplanted cells are differentiated, the more potential they have for migration and distribution through the cochlea.\textsuperscript{16}

The overall results of in-depth studies confirm that, independently of the SCs (ESCs, adult SCs or induced pluripotent stem cells [iPSCs]) or transplant technique used, several exogenous SCs can migrate extensively and survive in the cochlea in various animal models in which hearing loss is induced.\textsuperscript{16} Likewise, it has been shown that a great percentage of SCs express neuronal and glial markers after \textit{in vivo} implantation and can be conditioned to differentiate towards CHCs with functioning stereocilia and electromechanical activity.\textsuperscript{13,15} It has also been shown that the transplant generates minimum local tissue response.\textsuperscript{11,16}

Consequently, SGN regeneration or substitution would be one of the key points in restoring auditory function in sensorineural hearing loss and permitting auditory rehabilitation with future cochlear implants.\textsuperscript{1}

The next challenge in this process is to determine how to preserve the regenerated organ of Corti or cochlear ganglion from the immune system of the receptor.\textsuperscript{1}

Sources of Stem Cells for Use in Inner Ear Regeneration

Endogenous SCs can be induced to generate CHCs after an injury to the organ of Corti. However, generating neural elements to be used in otological transplants is a process that needs to be carried out in the laboratory from different types of cells (Table 3).

Endogenous Inner Ear Stem Cells

A population of SCs was discovered in human foetal cochlea.\textsuperscript{4} These cells preserve the expression of SC cell markers (Nestin, Sox2, Oct4 and Rex1). They can proliferate during several months and, when treated in special culture
conditions, present SGN and CHC characteristics; they also express neuronal differentiation markers such as Neurogen1, Brn3a, b-tubulin III and neurofilament 200.4

Embryonic Stem Cells

Inner ear progenitors have recently been generated from embryonic murine stem cells. These otic progenitors were differentiated in vitro, and a subpopulation showed a hair cell phenotype.9 When they were grafted in the inner ear of chicken embryos, they began expressing hair cell markers only when placed in sensory epithelium during development, not in other inner ear sites.9

ESC-derived neural progenitors have also been generated that can produce CH and SGN type cells when they are manipulated in vitro; these cells survive, differentiate and send projections towards the sensory otic epithelium in guinea pigs with hearing loss, demonstrating obvious functional recovery.4 However, performance is low and the techniques should be improved to increase the CHCs obtained from ESCs (0.36%),12 as well as to purify them of contamination from other ESC-derived cells. Mouse fibroblast-derived iPSCs can also be differentiated towards CHCs identical to those derived from ESCs, with an efficiency of 0.24%.12

Several work groups have used ESCs as in vivo substitutes for SGNs,12,13,19,20 obtaining the occupation of the space corresponding to the auditory nerve by neurites with efficient CHC-cochlear nucleus synapses. They were also able to restore the brainstem-evoked potentials.13

Adult Stem Cells

Adult SCs that have been used for inner ear regeneration are as follows: haematopoietic SCs from bone marrow, mesenchymal SCs and neuronal SCs.

Haematopoietic Stem Cells

Introducing cells from chinchilla bone marrow stroma has increased neuron and glial cell marker expression.7 Other authors used bone marrow-derived CD133+ SCs to produce cells with hair cell characteristics, using growth factors and forced expression of transcription factor Atoh1; however, the cells generated showed no integration or functional recovery.4,12 It is possible that these SCs act in a paracrine manner, helping in the regeneration process based on SCs endogenous to the ear.4

Mesenchymal Stem Cells

The attempt has been to obtain neurons from mesenchymal stem cells (MSCs); apart from differentiating into bone, cartilage, adipocytes and muscle, they also differentiated into in vivo and in vitro neurons and astrocytes.21

Advantages of MSCs include high expansion potential, genetic stability, ease of collection and shipment from the laboratory towards the hospitalised patient, ability to migrate to the injury site and a strong immunosuppressive property that can be exploited for autologous and heterologous transplants. One of their disadvantages is their lack of a specific marker that clearly defines the MSC phenotype. This phenotype variability limits their applicability, given that the functionality of the cell population obtained directly depends on the different sources and the extraction and culture technique.21

The best MSC source is bone marrow aspirate, although other sources are adipose tissue, liver, tendon, synovial membrane, amniotic fluid, placenta, umbilical cord and teeth. Some studies have successfully attempted to transfer MSCs to the cochlea to regenerate or replace cochlear fibrocytes. Despite not being sensory epithelium, they play an important role in cochlear physiology and gradually degenerate with age.22 The transplanted cells cannot differentiate to cochlear type cells spontaneously when they are mobilised from the bone marrow. Their effect is more one of protection through NTf secretion and modulation of inflammation.13 The adipose tissue-derived SCs have characteristics similar to the MSCs and could be obtained more easily.13

Further studies are needed if we want to use MSCs as a type of cell therapy for sensorineural hearing loss.21

Neural Stem Cells

Neural stem cells (NSCs), present in SNCs and peripher- al cells, possess little constituent cell renewal, but they can be recruited to substitute lost neurons and they have been successfully grafted in mouse inner ear.11,12,19 The transplanted cells survived several weeks, expressed neural markers of glial cells, neurons and hair cells9 and formed connections towards the CHCs in denervated cochleae.16 In the inner ear, cells with NSC characteristics have been discovered in vitro; however, it is unclear whether these cells are true NSCs or mesenchymal cells that contaminate the sample and are present in the inner ear stroma.12

Clinical applications for NSCs were demonstrated in several animal studies with SC or progenitor transplant to regenerate or recover a nerve lesion. However, the regeneration mechanism is unknown and it has not been established whether it is produced by (a) direct replacement of injured cells by transplanted cells, or (b) paracrine cytokine action, growth factors and hormones released by the transplanted cells and that act on the pre-existing cells. Such paracrine action has not been considered for a long time as the main mechanism of cellular regeneration. New studies are needed to establish the exact functional restoration mechanism.21

Neuron Generation Through Cellular Engineering

Cellular programming has partially addressed the practical difficulty of reaching endogenous inner ear SCs, the ethical problem raised by the use of human ESCs and the poor performance of adult SCs in other tissues towards an otic or neural phenotype. At present, engineering of somatic cells is the method of choice for generating almost all cell types, including sensory neurons. The versatility of this technique and its potential applications fully justify the fact that the
researchers Yamanaka and Gurdon received the 2012 Nobel Prize in Physiology or Medicine.

There are 2 ways that nerve differentiation can be obtained through *in vitro* engineering:

1. Engineering of somatic cells towards iPSCs and, from these, obtaining neurons. Differentiated adult cells are not a stagnant entity in the evolutionary process. This plasticity is due to silencing of genetic expression programmes that can be activated in cells differentiated by specific transcription factors, which allows them to fall back and advance in their cellular destiny and generate all cell types, including neurons, hematopoietic cells and cardiomyocytes. However, it is a difficult method, many times yielding variable and unpredictable results. The goal is generating iPSCs and, from these cells, imitate all the stages of differentiation, from neural progenitors to mature neurons. Fig. 3 presents the sequence for engineering of mononuclear human blood cells carried out in our laboratory using transfection with the Sendai virus. The iPSC colonies began to form on days 9–10. These colonies should expand for various weeks until their later characterisation and pluripotentiality validation. In a posterior stage, these iPSCs can be differentiated to hair cells or auditory neurons to develop an *in vitro* model of disease. Therefore, identifying candidate mutations in family forms of hearing loss, as occurs in Ménieré’s disease, will make it possible to obtain specific iPSCs with the mutations desired for investigating the molecular mechanisms of a disease.

2. Direct engineering of the somatic cell to a mature neuron. Generation of iPSCs has revolutionised the field of regenerative medicine and has established the bases for obtaining differentiated cell types. However, later studies have shown that it is possible to obtain differentiated adult cells by directly converting a differentiated adult cell into another adult cell from a different cell line. A combination of specific transcription factors or microRNAs is used to do so, without the cell having to go through the iPSC stage. What is attempted is forcing the over-expression of transcription factors that would activate specific genes in a mature neuron.

This has been achieved *in vitro* using the transcription factors Ascl1, Brn2 and Myt11. Combining these with the transcription factor NeuroD1 might make it possible to convert human fibroblasts into induced neurons with standard morphology and expression of neuronal markers, but with limited synopsis-generating capacity.

The cell sources used in inner ear experimentation are extremely varied and include dorsal root ganglion cells, neural progenitors, SCs or progenitors isolated from the inner ear, immortalised auditory neuroblasts, ESCs and bone marrow SCs. Our current cell differentiation knowledge makes it possible that SC research will continue in the majority of cases without resorting to ESC use.

**How to Insert These Therapies in the Cochlea?**

The development of new techniques to apply these advanced therapies for the cochlea (SC transplant, gene therapy and drug or trophic factor insertion) is still essential for them to be efficient.

Transplanting exogenous SCs to the cochlea and having them migrate towards Rosenthal’s canal (RC), where the SGNs are located, is a difficult technique. The cochlear structure limits cell migration and surgical access to the cochlea can result in severe hearing loss.

Different routes have been attempted for the transplant. An example is the intra-perilymphatic and intradendympathic paths, which are more accessible but yield low cell survival and little migration towards the RC. Another example is using the modiolus or auditory nerve trunk, which leads to greater survival and cell migration, but with the possibility of causing severe hearing loss. Cells transplanted to occupy the RC space have more nerve projections towards the CHCs and cochlear nuclei that those placed in the eardrum or middle ear. Because the ideal pathway has yet to be defined, alternate routes are still being investigated. There are authors that are in favour of using the lateral cochlear wall as the pathway for cell transplants because of its abundant tissue and vascular irrigation (which promote transplant survival), as well as the low possibility of injuring the cochlear structures. The lateral cochlear wall pathway...
offers better rates of cell survival and RC-oriented migration than other approaches. This migration, like others, can be optimised by using chemotactic molecular factors concomitantly.28

The performance of the technique could be improved by using a microinjector or an osmotic pump coupled with a catheter system to allow multiple injections of small cellular volumes in specific cochlea sites. Developing specialised instruments, such as microendoscopes or inoculation systems associated to the cochlear implant, are other options for drug inoculation, gene therapy and progenitor cell transplantation.7

Despite the variety of techniques, the limiting factors in transplants are still the difficulty of appropriate integration in the complex structure of the organ of Corti (narrow cell junctions) and the possible loss of endolymph from the cochlear duct from the surgical procedure.7

Conclusions

1 Engineering of human iPSCs obtained from the patient him/herself is the safest method for regenerating sensorineural hair cells and cochlear and neurons.

2 Activating endogenous SCs by gene overexpression or inhibition through gene or drug therapy is a very promising therapy.

3 Combining several techniques with a synergic effect (such as cochlear implants with SCs, gene therapy and drug treatments) seems to be the therapeutic strategy that could produce the best medium-term results.

4 Personalised application of the therapies, based on the cochlear and neuron reserve of each patient, is essential.

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Conflict of Interests

The authors have no conflicts of interest to declare.

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