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Advancements in Stem Cell Technology and Organoids for the Restoration of Sensorineural Hearing Loss

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Abstract

Background Sensorineural hearing loss (SNHL) is a significant cause of morbidity worldwide and currently has no curative treatment. Technological advancements in stem cell therapy have led to numerous studies that examine the generation of otic sensory cells from progenitors to restore inner ear function. Recently, organoids have emerged as a promising technique to further advance the process of creating functional replacement cells after irreversible hearing loss. Organoids are the three-dimensional generation of stem cells in culture to model the tissue organization and cellular components of the inner ear. Organoids have emerged as a promising technique to create functioning cochlear structures in vitro and may provide crucial information for the utilization of stem cells to restore SNHL.

Purpose The purpose of this review is to discuss the recent advancements in stem cell-based regenerative therapy for SNHL.

Results Recent studies have improved our understanding about the developmental pathways involved in the generation of hair cells and spiral ganglion neurons. However, significant challenges remain in elucidating the molecular interactions and interplay required for stem cells to differentiate and function as otic sensory cells. A few of the challenges encountered with traditional stem cell therapy may be addressed with organoids.

Conclusion Stem cell-based regenerative therapy holds a great potential for developing novel treatment modalities for SNHL. Further advancements are needed in addressing the challenges associated with stem cell-based regenerative therapy and promote their translation from bench to bedside.

Keywords
- hearing loss
- stem cells
- organoids
- otic sensory cells

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Hearing loss is one of the most common neurosensory deficits among children and adults worldwide.[1] Sensorineural hearing loss (SNHL) affects approximately 25 to 48 million Americans with approximately 1 in 1,000 children having profound to severe SNHL.[2] SNHL is caused by damage to the auditory hair cells that line the sensory compartment of the cochlea. Hair cells are sensory cells that are responsible for transducing mechanical stimuli, such as sound and movement, into an electrical stimulus, which then travels to the auditory cortex of the brain via spiral ganglion neurons (SGNs).[3] Both hair cells and SGNs are unable to regenerate or self-repair, therefore, damage to these sensory structures leads to irreversible hearing loss. There are many different etiologies responsible for the permanent damage to otic sensory cells including congenital defects, ototoxic medications, and exposure to loud noise.[4]

Stem cell therapy is an exciting and promising approach to restore sensory cells that are damaged in SNHL. Research has suggested the possibility of restoring sensory cells for hearing and balance with the use of in vitro differentiation of stem cells into hair cells or auditory neurons. The utility of stem cell-based therapy for SNHL is dependent on the ability of transplanted cells to not only survive and migrate to the appropriate damaged areas of the cochlea, but also successfully transduce sound to the brain. Many studies describe the differentiation of stem cells into inner ear progenitor and sensory cells using various types of stems cells, mammalian models, and methods of differentiation.[4] Despite these advancements, many limitations prevent these techniques from being used in the clinical setting.

Another promising area of interest in SNHL treatment is the development of organoids. Organoids are three-dimensional (3D) stem cell cultures that can function as an external cellular environment and serve as a powerful tool to study the pathways involved in both SNHL and generation of otic sensory cells.[5] Though organoids are a relatively new technology with their own limitations, they address some of the pitfalls that hinder traditional methods of stem cell culturing. In this review, we will discuss the utilization, success, challenges, and possible future directions of both stem cell therapy as well as organoids for the treatment of SNHL.

Principles of Inner Ear Stem Cell Therapy

Stem Cell Types and Delivery

Stem cells are characterized by their ability to differentiate into various cell types and are classified by the lineages they descend from[6] (►**Fig. 1**).[7] The stem cells used in studies involved with SNHL include embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) (►**Fig. 3**), and

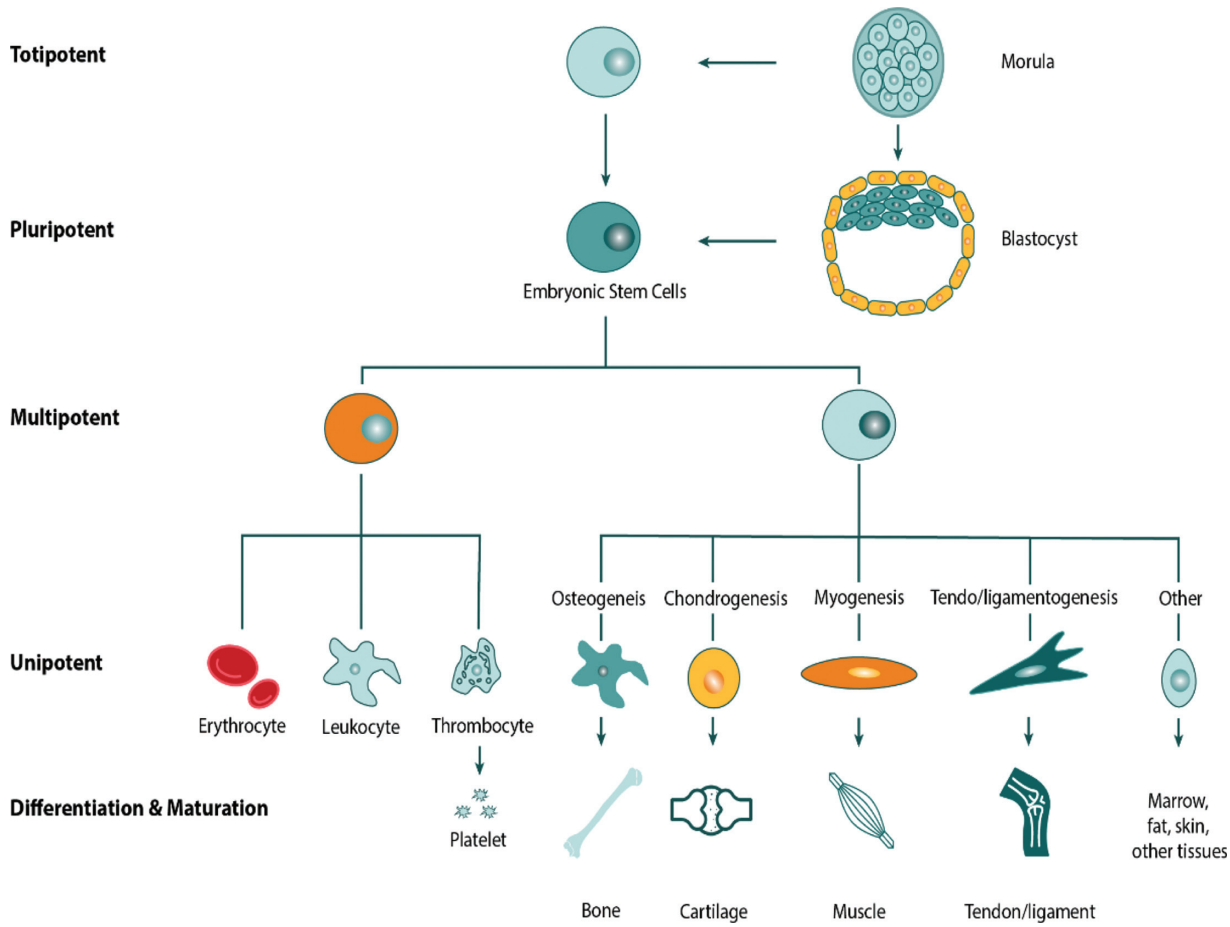

Fig. 1 *Stem cell lineage.* Stem cells have the ability to differentiate into specific cell types. There are three major classes of stem cells: totipotent cells that can give rise to any cell type or form an embryo, pluripotent cells that can become any cell in the adult body, and multipotent cells that are restricted to becoming a more limited population of cells. Adapted from Zhang et al 2009.[7]

mesenchymal stem cells (MSCs). ESCs are derived from an embryo during the blastocyst stage and can differentiate into both hematopoietic and mesenchymal cell lineages. All tissues can descend from these two cell lines that gives ESC their pluripotent potential. iPSCs have the same capacity to differentiate into any tissue but are derived from somatic cells. These somatic cells, such as fibroblasts, usually come from adult tissue and are induced to dedifferentiate back into pluripotent cells. While ESCs and iPSCs are both pluripotent, MSCs, which are multipotent cells, are only capable of differentiate into certain types of tissue. Though easily isolated, MSCs are limited by a lack of proven efficacy in differentiating and functioning as specialized cells [6] Each type of stem cell mentioned above has been studied to evaluate their efficacy in becoming functional otic sensory cells that can replace the damaged tissue responsible for SNHL.

The Delivery of Stem Cells into the Cochlea

A major hurdle to overcome in the use of stem cell therapy for hearing loss is successfully delivering in vitro generated cells into the cochlea (►**Fig. 2**).[8] Three main techniques have been investigated for stem cell delivery to the inner ear; systemic administration, trans-tympanic administration, and intracochlear administration (►**Table 1**). The tightly regulated blood–labyrinth barrier (BLB) separates the inner ear from systemic blood circulation, seemingly separating the two structures from cellular migration. However, MSCs have demonstrated the ability to migrate to SGNs and hair cells within the cochlea when they are injected into the brachial vein of guinea pigs.[9] These findings suggest MSCs are able to penetrate highly regulated barriers, such as BLBs, and potentially the round window membrane (RWM), to reach the cochlea and migrate to the area of neuronal damage.

Trans-tympanic administration of stem cells has also shown promising results. Injection of stem cells through the tympanic membrane and into the middle ear allows for the stem cells to be in close proximity to the cochlea. Stem cells are traditionally thought to drive much of their regenerative potential from direct replacement and repair of injured tissues. However, there is an increasing body of evidence that shows the paracrine signaling between grafted stem cells and host tissue can be otoprotective.[10] In this way, stem cells can be injected in close proximity to the inner ear and still have an effect without being directly injected into the inner ear. In fact, many studies have observed the beneficial therapeutic effects of stem cell administration despite the failure of cells to engraft within the target tissues.[11] Additionally, studies have demonstrated that stem cells secrete many biologically active molecules without engraftment: insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor-D (VEGF-D), and interleukin-

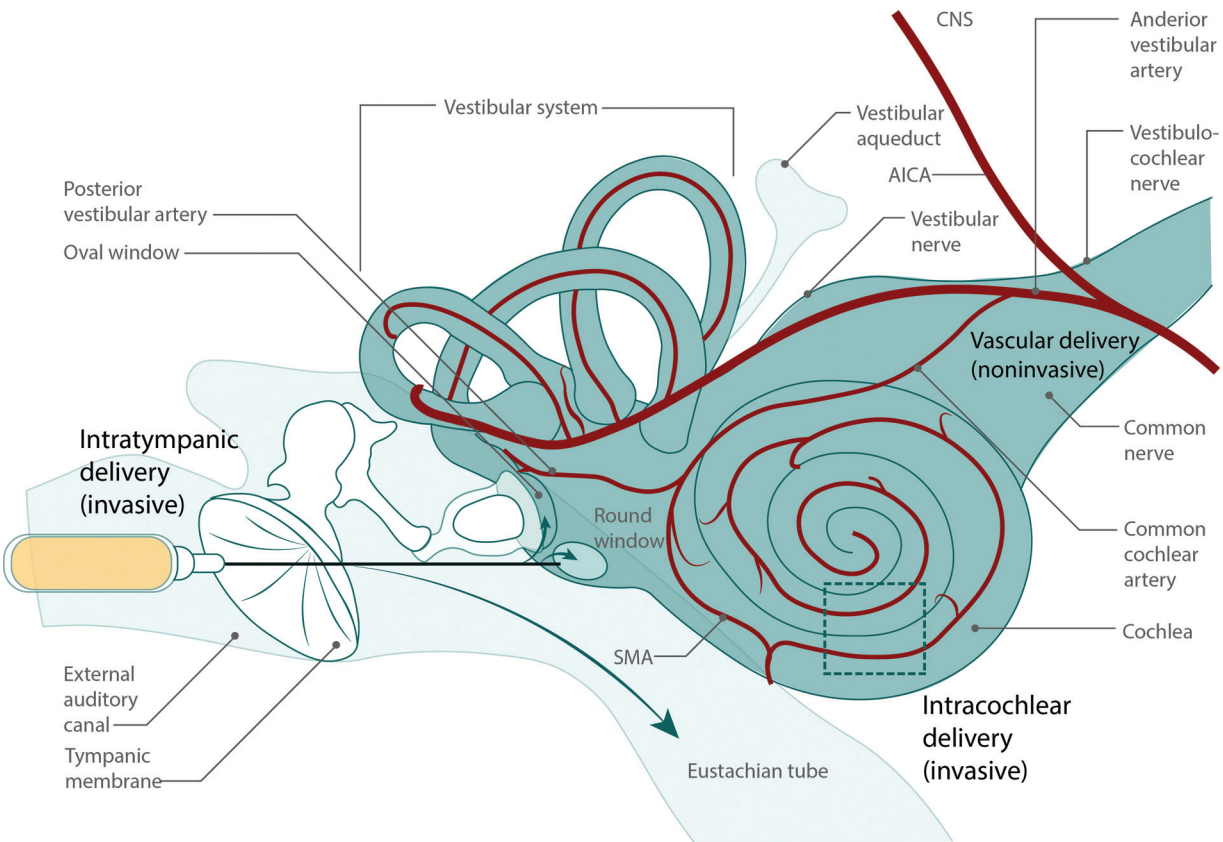

Fig. 2 *Stem cell delivery to the inner ear:* The inner ear is located behind the tympanic membrane and can be approached with an incision in the tympanic membrane. Although the inner ear is surrounded by bone, this area is filled with endolymph and perilymph fluids. Mechanical and hydraulic damages to the inner ear as a result of injection of stem cells directly into the cochlea may lead to further hearing loss. Conversely, systemic administration of stem cells through vasculature may have other type of side effects (stem cells must traverse the blood–labyrinth barrier or may reach other unwanted organs). Adapted from Nyberg et al 2019.[8]

Table 1 A summary of various stem cell delivery techniques

Mode of delivery	Advantages	Disadvantages
Intratympanic	• Anti-inflammatory paracrine signaling without direct engraftment. • Rapid and proximal delivery	• Difficulty reaching target locations due to limitations of the round window membrane permeability.
Intracochlear	• Most direct route of administration. • Can bypass protective barriers.	• Invasive Only performed in conjunction with surgery (i.e., cochleostomy or round window incision and insertion of catheter) High risk for damaging adjacent structures.
Vascular/ Systemic	• Therapeutics can be developed to cross the blood–labyrinth barrier. Drug delivery vehicles may reduce the chance of off target delivery.	Off-target delivery to locations with similar cell surface markers leading to adverse reactions. Lack of full understanding of the cochlear microcirculation.

10 (IL-10).[12] In one study looking at hypertrophic scar formation and wound healing, the paracrine signaling of various factors from transplanted MSCs showed anti-inflammatory, antiapoptotic, pro-mitotic, and angiogenic properties.[13] This combination of signaling may garner an otoprotective effect that potentially helps hair cells and SGNs to regenerate.

Intracochlear administration of stem cells is by far the most direct route of stem cell administration to the inner ear. Intracochlear injection is beneficial due to the precise delivery of stem cells into the cochlear space. This allows cells to bypass many of the barriers protecting the inner ear. Unfortunately, the use of these injections is currently limited in vivo due to the risk of damaging sensitive local structures during injection.[14] This limitation relegates intracochlear injections to potentially only cases of profound hearing loss. However, one promising development has been the creation of biohybrid cochlear implants. Biohybrid implants coat the electrode array with stem cells prior to insertion in the hopes of preserving residual hearing post implantation because the stem cells release cytokines, chemokines, and growth factors that exert anti-inflammatory and neuroprotective effects.[15] These effects in turn reduce fibrosis and cochlear ossification thereby potentially increasing the residual SGN counts.[15]

Stem Cell Therapy in the Inner Ear

Stem cell therapy can be divided into three categories based on cell type: ESCs, adult-derived stem cells (ASCs), and induced pluripotent stem cells (iPSCs) (►**Fig. 3**). Each of the different cell types within this framework provides distinct advantages and disadvantages.

Embryonic Stem Cells

As previously mentioned, ESCs display unlimited capacity to proliferate and can differentiate into any tissue type. This aspect makes them attractive for cellular therapy but highly controversial since they need to be isolated from embryos. Many studies have observed exciting results using animal ESCs to derive otic sensory cells (►**Fig. 3**). A study developed a protocol to guide differentiation of mouse ESCs to otic sensory neurons (OSNs) using bone morphogenetic protein (BMP) signaling and transforming growth factor-β (TGF-β) signaling inhibition. The study successfully derived a population of neurons that expressed neuronal morphology and functional properties of OSNs.[16] Another study also developed a protocol to generate otic sensory cells by treating human ESCs with various proteins such as human bone morphogenic protein-4 (BMP4), wingless-related integration site 3a (Wnt3a), fibroblast growth factor-2 (FGF-2), epidermal growth factor (EGF), and mitogenic factors. The induced cell population was functionally and phenotypically similar to spiral ganglion cells.[17]

A study generated early primitive ectoderm-like (EPL) cells by culturing mouse ESCs in a conditioned medium. Subsequently, the ESC-derived EPL cells were induced with a combination of FGF3, FGF10, EGF, and IGF-I to generate otic progenitor cells. The induced cells developed stereociliary hair bundle-like structures and contained functional mechanotransduction channels.[18] Functional recovery of auditory neurons in vivo has only been demonstrated in one study with ESCs. The successful restoration of inner ear function was observed following transplantation of human embryonic-derived otic progenitor cells into mice with deafness induced by Ouabain treatment. In this study, there was a detectable improvement in the auditory brainstem response (ABR) thresholds of transplanted animals starting at 4 weeks post transplantation, with the mean auditory threshold improving to 50.46 dB by 10 weeks post-transplantation. The transplanted mice were observed to have an overall functional improvement of 46% with some of the animals experiencing near total restoration of hearing.[19]

Adult-Derived Stem Cells

Adult stem cells do not pose the same ethical issues as seen with ESCs; however, they lack the same degree of pluripotency because they are more differentiated into a particular tissue type. MSCs have been used in experiments looking at SNHL due to their low side effect profile and relatively straightforward mode of isolation. A phase 1 clinical trial was conducted to evaluate the intravenous administration of human umbilical cord blood (hUCB) in children with SNHL. The primary objective of the study was to evaluate the safety and feasibility of cord blood autologous transplants as well as

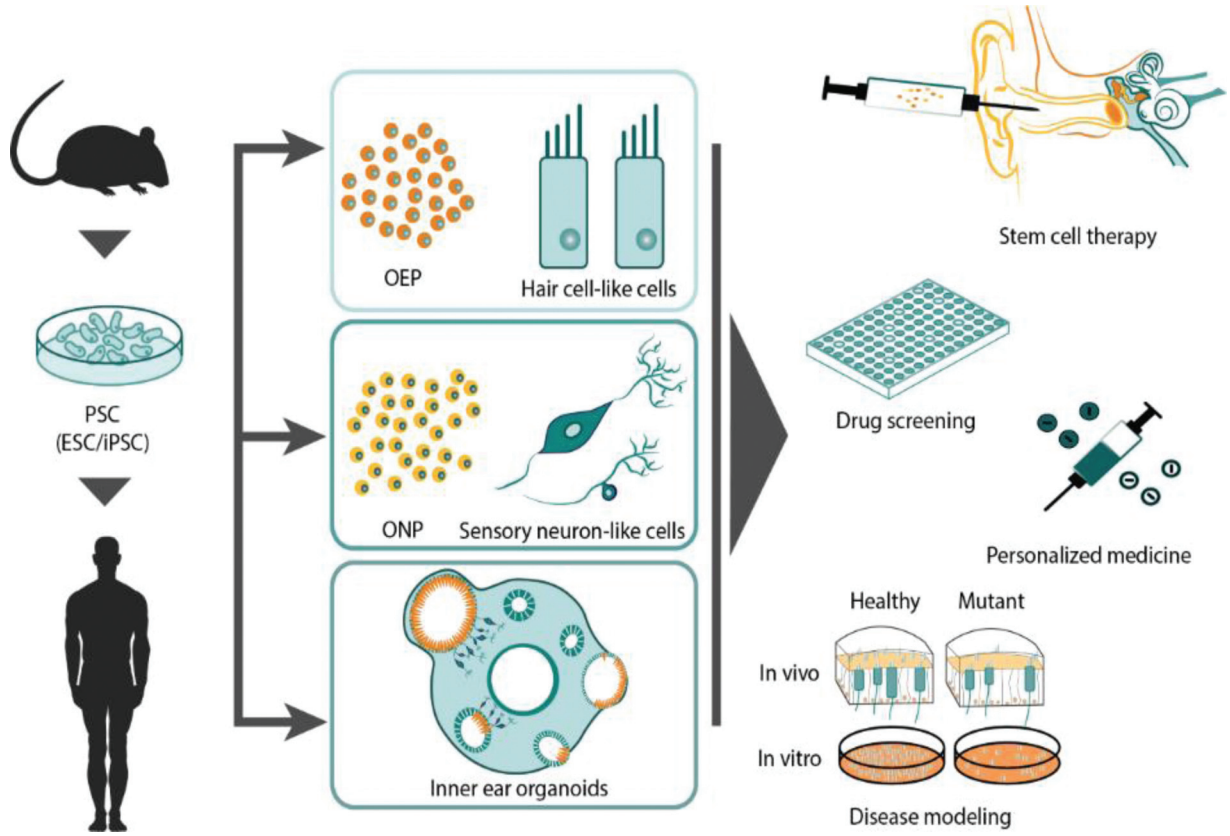

Fig. 3 : *Use of inner ear stem cells:* Human and rodent embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) can be induced to form otic epithelial progenitors (OEPs) that develop into hair cells, otic neural progenitor (ONP) cells that develop into sensory neuron-like neurons, and 3D inner ear organoids which can be used to model and experiment with healthy and diseased inner ear structures. Adapted from Tang et al 2020.[4]

to determine functional and structural improvements in hearing. Results showed five of eight subjects experienced a reduction in ABR thresholds after transfusion. The reduction in ABR threshold was evident throughout a follow-up period of 12 months. Additionally, fractional anisotropy (FA), a proxy for myelination, and white matter quality usually decreased in individuals with SNHL, was measured. Out of the five patients who had improved thresholds, three presented with improvement in FA, suggesting possible improvement in nervous system structures associated with the auditory pathway.[20] A case study reported a patient with SNHL who underwent an autologous bone marrow-derived MSC transplant for hearing loss regeneration. The cells were transfused through a subclavian line and then homed to the cochlea by applying electrical stimulation to the promontory. Electrical stimulation has previously been established as a helpful tool for cardiac human stem cell differentiation and brain stimulation.[21] The researchers hypothesized that this technique would aid in localizing the stem cells to the damaged cochlea. Although the patient did not experience any complications or side effects, the authors were unable to observe any change in ABR thresholds up to 1 year.[22]

Besides MSCs, there has been increased interest in dental pulp-derived stem cells for developing therapeutic modalities for hearing loss. A study aimed to use stem cells derived from dental pulp and deciduous teeth and differentiate them into SGNs using neurotrophins.[23] Dental pulp cells are close in origin to ESCs but lack the ethical issues with isolation. Using basic fibroblast growth factor and EGF, the study induced the stem cells into neuronal differentiation. The cells demonstrated long neuronal processes with spherical bodies and expressed SGN-specific markers.[23]

Induced Pluripotent Stem Cells

iPSCs can be derived from an autologous source similar to adult stem cells, however, when these cells are induced, they have pluripotent potential. A study transplanted mouse-derived iPSCs into mice cochleae. After 4 weeks, the injected stem cells were observed to differentiate into hair cell-like cells and SGN-like cells. Although there was a significant difference in ABR thresholds between the treated mice and controls, the mice still exhibited severe hearing loss following transplantation.[24] Another study isolated urinary cells from a healthy human donor and dedifferentiated them into iPSCs.[25] The iPSCs were then induced to become otic progenitor cells, which were observed to further differentiate into hair cell-like cells. These cells were analyzed for their potential to synapse with SGNs in vitro and transplanted into mice cochleae in vivo for assessment of engraftment. The results of this study showed afferent synaptogenesis between hair cell-like cells and SGNs and successful migration of cells into the scala media and scala tympani. Furthermore, some of the stem cells migrated to the site of resident hair

cells and demonstrated differentiation into hair cell-like cells.[25]

Inner Ear Organoids

Organoids were developed initially as mimics for gut and brain tissue, organoids are 3D cell cultures derived from stem cells that are representative of a larger organ in regards to cell types and function.[26] By containing multiple cell types and proper spatial arrangement, 3D organoids are able to better represent their target organ compared with two-dimensional culture methods.[27] Organoids provide an unprecedented opportunity to expand existing knowledge of the inner ear and improve current treatment modalities. Many of the problems encountered in inner ear research and stem cell therapy are hoped to be addressed with organoids.

Organoids can model the human inner ear better than traditional animal models that use zebrafish and rodent species. While these models have been invaluable in developing our current understanding about the inner ear, there are several anatomical and molecular differences between animal models and the human inner ear. For example, murine cochlear explants, a commonly used inner ear model, are not mature at birth, while human cochleae are fully mature by the 20th week of embryogenesis.[28] Additionally, murine cochleae must be dissected before post-natal day seven and can only be kept ex vivo for approximately 2 weeks.[4,29,30] Other animal models such as zebrafish have more hair cells and a higher capacity for spontaneous hair cell regeneration.[31,32] Despite these advantages, zebrafish do not accurately represent the human inner ear; access to the inner ear in humans is highly restricted by the surrounding bone, and attempted access can cause damage to the fragile structures within the inner ear, significantly limiting in vivo sampling. Additionally, noninvasive imaging techniques do not provide the level of detail needed to study inner ear pathology.[4,33] Organoids overcome these issues, as they are more easily accessible, sustainable, and modifiable, all while more closely recapitulating the human cochlear function.

Additionally, unlike other animals, the mature human cochlea cannot spontaneously regenerate both sensory and neural cells after damage. This lack of innate reparative ability makes the concept of assembling complex cellular structures in 3D particularly appealing. A study induced the differentiation of human-induced pluripotent stem cells (hiPSCs) into otic progenitors to form SGNs and hair cells using organoid techniques. The generated cells were then injected through the RWM and after 4 weeks, were observed to migrate past the membrane that contained perilymph and endolymph. Some of the transplanted cells migrated into the scala media, however once there, they failed to transform into hair cells. Furthermore, only a few cells were identified to have integrated to the site where hair cells were originally lost.[25] The findings from this study were consistent with the previous study that transplanted hiPSCs through the round window niche. Results from this study showed that 4 weeks after transplant, the cells expressed auditory hair cell markers and SGN markers.[34] A study performed in vivo transplantation of inner ear organoids in an adult mammal. The high potassium level in the endolymphatic compartments can easily damage or kill transplanted stem cells thus the researchers injected the cells into the scala tympani at the basal turn by cochleostomy, which maintained an intact bony rim between the injection point and the round window. Cyclosporine was used as a pretreatment to induce immunosuppression and it was observed that the grafted cells localized preferentially within damaged regions of the cochlea. It was also demonstrated that hair cell progenitor cells engrafted into the cochlea and were able to survive as cochlear sensory epithelium.[1]

Use of PSC built organoids to replace and repair damaged sensory epithelium and neurons could potentially be curative for these conditions. Indeed, thus far, experiments have shown that transplantation of early PSC-derived otic progenitors has been successful, although the long-term viability and function of these transplants remain unclear.[4] Currently only a few early-adopting laboratories are investigating the potential of organoids. More widespread investigation and use of these novel experimental modalities are needed to increase our understanding about human inner ear pathophysiology and potentially develop treatment modalities for inner ear disorders. To this end, we will discuss the current state of organoid research.

Organoid Creation

Organoids are human pluripotent stem cells (hPSCs) which are cultured in a way to replicate in vivo embryological development (►**Fig. 4**).[35] To begin, hPSCs are aggregated in low-binding plates treated with extracellular matrix proteins (Matrigel) to promote epithelialization of the surface. Next, a series of key growth factors that control molecular signaling pathways are introduced in a stepwise fashion, such as TGF, FGF, Wnt, and BMP (►**Fig. 5**).[36] This series of signaling molecules induce the formation of non-neural ectoderm (NNE), otic-epibranchial progenitor (OEP) domain, and otic placodes in sequential order. The otic placodes then self-differentiate into otic vesicles that evolve to form sensory epithelia consisting of hair cells, support cells, and neuronal cells needed for innervation.[28,37,38] This process is similar to those used to make cerebral and retinal organoids with the crucial branch points being the TGFβ, BMP signaling-induced NNE formation, and BMP and FGF signaling-induced otic placode formation.[39] Additionally, the physical cues provided by the extracellular matrix are vital to the efficacy of differentiation and assembly into the hair cell lines vesicles.[37,40,41]

The most groundbreaking step in the creation of inner ear organoids was pairing both the mechanosensory cell lines and neuronal cell lines within the same model system to replicate the mechanism of sound transduction in vitro. These two systems are intimately related in transducing sound yet were previously generated separately in model systems.[4] Indeed, these models have already been used to investigate inner ear physiology and disease.[42,43] However, despite these advances, further characterization of these systems is still needed.

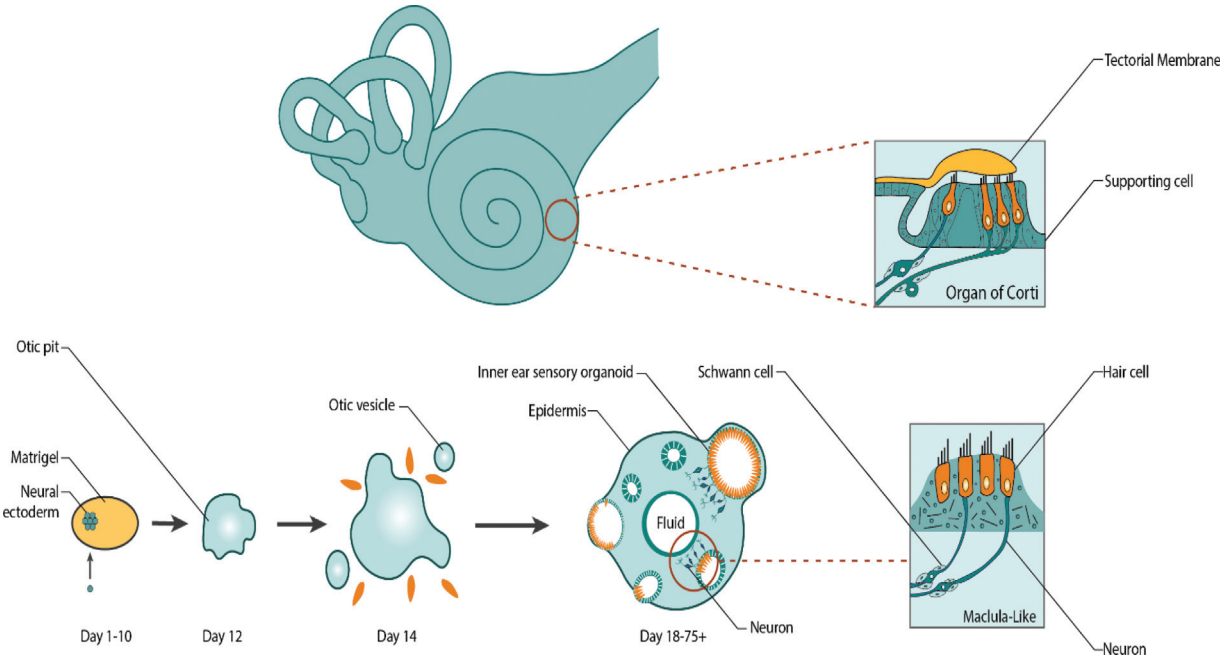

Fig. 4 *Inner ear organoids:* both guided differentiation and spontaneous self-organization in three-dimensional cultures have allowed for the successful generation of otic vesicle-like structures containing functionally mature sensory hair cells from mESCs. The use of extracellular matrix proteins and epithelial support cells allows for an extracellular matrix and supportive cellular environment in which signaling molecules can function to control stem cell proliferation and differentiation. Adapted from Munnamalai and Fekete 2017.[35]

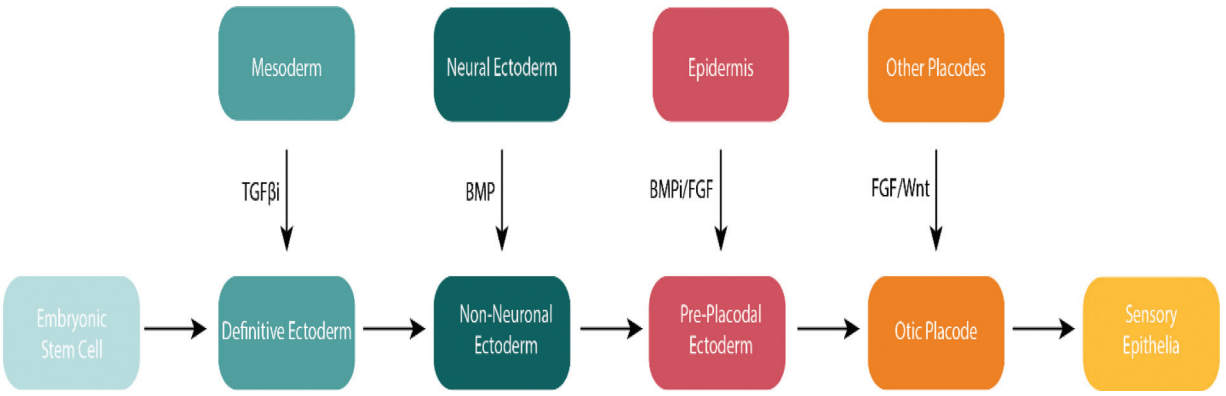

Fig. 5 *Steps to generation of sensory epithelium:* Key signaling mechanisms guide the process from embryonic stem cell to definitive ectoderm and eventually to sensory epithelia. A TGF-β inhibitor blocks formation of mesoderm/endoderm and promotes induction of definitive ectoderm while BMP signaling is used to induce non-neural ectoderm. BMP inhibition and FGF activation are necessary for the development of pre-placodal ectoderm which with subsequent FGF signaling and endogenous Wnt become an otic placode. Adapted from Longworth-Mills et al 2016.[36] BMP, bone morphogenetic protein; FGF, fibroblast growth factor; TGF-β, transforming growth factor-β.

Additionally, this process is not without limitations. Researchers report that the efficiency of organoid culture can be affected by stem cell passage number, variability in the number of reagents, seasonal variations, as well as variations in dose and timing within the protocol.[39] For example, the extracellular matrix, Matrigel, as a biological product, has a high degree of variability and unpredictability between lot numbers; however, there is currently no effective alternative to mitigate these challenges.[44]

Experimental and Therapeutic Use of Organoids

As mentioned previously, organoids present an entirely new experimental paradigm with which researchers can investigate cochlear function and disease, as well as a large step forward in using stem cells as treatment modalities in the inner ear. The use of inner ear organoids to investigate these avenues is particularly attractive given the potential ease and scalability of the experimental design.

Drug Identification and Screening

Cochlear organoids have a promising role in drug development and discovery. Current testing pipelines for otologic drugs have challenges since they rely heavily on animal models to test the safety and efficacy of drugs. The organ of Corti explants used in current regenerative studies are from generally P7 post-natal animals which mean they still have immature cochlea. In addition, these explants may have a significantly different capacity for cellular regeneration and

toxicity compared with adult rodents as well as to their human counterparts.[28,45] If the experiment is performed in vivo, it is then highly limited in throughput due to feasibility issues as it is difficult to handle large number of animals at the same time. Even high throughput in vivo models such as zebrafish are limited by their physiological differences compared with mammalian hearing.[27] Given these limitations, the use of human-derived cochlear organoids is particularly appealing and can serve as a powerful tool for developing regenerative therapies for hearing loss.

Inner Ear Regenerating and Repair

Finally, human-derived organoids are being studied in the context of direct stem cell therapy. Current research into inner ear stem cell therapies face several problems, especially regarding adequate engraftment into target tissues and adequate function once administered.[28,46] The ability to manipulate cells post transplantation into damaged cochleae is a significantly challenging area for sensory regeneration. Barboza et al harvested inner ear progenitor cells from mice and transplanted them into guinea pigs with neomycin-induced SNHL. Following transplantation, the cells were found in all scala of basal turns. Of the transplanted stem cells in the scala media, $42.6 \pm 5.7\%$ were positive for the essential hair cell marker MYO7A.[47] It is theorized that the level of differentiation of donor cells may affect the subsequent rate of engraftment of cells into target organs. For example, research has revealed that stem cells grafted at lower levels of differentiation are more at risk for tumorigenesis, while cells grafted at moderate levels of differentiation have a lower survival rate.[48,49]

Despite advances in delivery technique and technology, very few stem cells migrate appropriately, and even fewer of those survive after intracochlear injection.[50,51] These issues in cell survival and differentiation partially derive from the inaccessibility of the inner ear structures to stem cell placement. Cell survival is also affected by the toxic environment created by the endolymph.[4] Moreover, these transplanted cells frequently struggle to differentiate into hair cells and neurons, rendering them functionally useless.[52] Organoids will help to solve this problem by delivering more differentiated otic neural progenitors (ONPs) and OEPs into the target tissue. In experiments injecting human-derived OEPs into gerbil cochlea, cells demonstrated migration, engraftment, and differentiation. Furthermore, they were able to form synapses with sensory neurons. In this study, auditory functioning post-transplant was not assessed.[53] While this is a very promising finding, much more research is needed to investigate the functionality of organoids and to increase the number of synaptic connections within the SGN.

Challenges with Regenerative Stem Cell Therapy

Stem cell-derived therapy is associated with limitations that pose a significant barrier in its potential translation from bench to bedside. Once cells are successfully transplanted into the cochlea, besides from functioning properly, they must exhibit biocompatibility. In a study, guinea pigs were transplanted with mouse stem cells. Although an immunological rejection would seem likely due to the differing species, the researchers did not see any evidence of infiltration by inflammatory cells or other signs of rejection.[47] Other studies have observed similar findings with xenologous transplantations, which may indicate that the cochlea is an immune-privileged organ similar to the eye.[48,54] On par with these findings, we also observed that MSCs are biocompatible with the cochlea in a rat model. There was no evidence of an immune reaction or inflammation throughout the adjacent structures, cochlea, or middle ear on histological examination following transtympanic administration of bone marrow-derived MSCs to rats without immunosuppressive therapy. The results of our study suggest the possible efficacy of MSCs as a modality of treating SNHL without immunological complications.[55]

Previous studies have also reported the potential association between transplantation of stem cells and higher risk of tumor formation particularly after the administration of undifferentiated stem cells.[56] In the study previously mentioned by Chen et al, no tumor formation was detected in the mouse cochlea 4 weeks following transplant.[34] However, another study also evaluated the tumorigenesis risk of iPSCs and found that after transplanting the iPSCs derived from different somatic cells into mice, the iPSCs derived from adult mouse tail-tip fibroblasts had a higher risk of tumorigenesis than iPSCs cells from mouse embryonic fibroblasts.[57]

Despite advances in delivery technique and technology, very few stem cells migrate appropriately, and even fewer of those survive after intracochlear injection.[50,51] These issues in cell survival and differentiation partially derive from the inaccessibility of the inner ear structures to stem cell placement. Cell survival is also affected by the toxic potassium-rich environment of the endolymph.[4] Moreover, these transplanted cells frequently struggle to differentiate into hair cells and neurons, rendering them functionally useless.[52] Organoids will help to solve this problem by delivering more differentiated ONP and OEP into the target tissue. In experiments injecting human-derived OEPs into gerbil cochlea, cells demonstrated migration, engraftment, and differentiation. Furthermore, they were able to form synapses with sensory neurons. However, in this study, auditory functioning post-transplant was not assessed.[53] While this is a very promising finding, further studies are warranted to explore the regenerative potential of organoids, to increase the number of synaptic connections within the SGN, and to evaluate the potential of providing functional benefits.

Conclusion and Future Directions

SNHL is a prevalent condition that causes significant morbidity for millions of individuals around the world. There is currently no curative treatment for SNHL and the therapeutic interventions available, including hearing amplification devices, cochlear implants, and other implantable devices, are often expensive and inaccessible to a large proportion of the population. For these reasons, the scientific community has been pushed to explore stem cell therapy for the restoration of inner ear function. Many

studies and preclinical models have demonstrated the potential for stem cell therapy to address the permanent hearing loss associated with SNHL, while simultaneously revealing the large leaps the research community still needs to make. One area that must continue to develop is the route and mode of delivery of stem cells to the inner ear. Studies have revealed the difficulty of administering stem cells into the organ of Corti and ensuring localization of the cells to damaged areas for engraftment.

The various types of stem cells also pose clinical challenges. ESCs have pluripotent potential and demonstrate an ability to differentiate into otic sensory cells and improve hearing function. Despite the success observed in preliminary studies that utilized this cell type, stem cells derived from embryos pose technical and ethical difficulties. Adult-derived stem cells are an attractive choice due to their relative ease of collection and ability to be used by patients themselves for autologous transplant. This advantage makes them more accessible, but due to their later state of differentiation, they lack the same pluripotent potential as ESCs. IPSCs address the issue of potency since adult stem cells can now be dedifferentiated into pluripotent cells and then redifferentiated into specific otic sensory cells.

Despite these advancements, further research is necessary to characterize the differentiation pathways involved in restoring inner ear hair cell function with stem cells. Wnt, β-catenin, and Notch are established important signaling pathways in the generation of hair cells, but the numerous cross-interactions and molecular interplay that are also necessary have yet to be fully elucidated.[58] The development of organoids is an exciting opportunity to address many of the challenges faced with traditional stem cell techniques. Organoid models closely recapitulate the inner ear organization and cellular composition and therefore well suited for the study of genetic defects associated with SNHL. Furthermore, organoids provide a reliable model to test toxicity of drugs on otic sensory cells. Importantly, though studies have shown minimal complications and side effects, stem cell therapy has the potential to cause inflammatory reactions, including transplant rejection and tumorigenesis. It is imperative that the safety of stem cell therapy is fully explored before clinical use. Rapid technological advancements in the research hearing community make stem cell technology and organoid cultures a promising future treatment modality for SNHL.

Conflict of Interest
None Declared.

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