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Review Article

Strategies for Stem Cell-Based Therapy for Inner Ear Cochlear Regeneration

Ali Asghar Peyvandi[1], Hojjat-Allah Abbaszadeh[2,1,3], Shahrokh Khoshsirat[1], Alireza Zali[4], Somayeh Niknazar[4,1*]

1. Hearing Disorders Research Center, Loghman Hakim Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Laser Application in Medical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Anatomical Sciences and Biology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
4. Functional Neurosurgery Research Center, Shohada Tajrish Comprehensive Neurosurgical Center of Excellence, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ABSTRACT

The organ of Corti of mammals has an organized structure in which row of inner and outer hair cells (HCs) are enclosed within the numerous cells on the basilar membrane. Given the prevalence of sensorineural hearing loss due to aging and acoustic insult, it is highly desirable to develop a protocol that produces cochlear sensory cells and their associated spiral sensory neurons as a tool to advance understanding of inner ear development. The replacement of damaged auditory neurons holds promise for significantly improving clinical outcomes in deaf patients. Cell therapy is one of the treatment options for deafness. The progress in cell therapy and reprogramming techniques has opened avenues to stimulate either endogenous or transplanted stem cells, aiming to replace and repair damaged inner ear HCs and restore auditory function. In fact, current research focuses on generating functional HCs. Various approaches are being explored to regenerate auditory HCs and facilitate neural connections. Here is an overview of existing experimental culture setups for the HCs and auditory neurons regeneration and their potential treatment for hearing disorders.

Keywords:
Cell therapy
Hair cells
Spiral ganglion neurons
Regeneration

Introduction

Hair cells (HCs) and auditory neurons in the inner ear work together to send acoustic information to the brain (Hyakumura et al., 2019). A complex cell arrangement during embryonic development in a precisely coordinated spatiotemporal manner is required for proper function of the inner ear of vertebrates. HCs are located in both the auditory and vestibular regions of the inner ear (Menendez et al., 2020). The primary cause of deafness often stems from the death and/or dysfunction of HCs within the organ of Corti (Khoshsirat et al., 2021). HCs are precisely arranged, with a single row of inner HCs on the medial side of the epithelium and three rows of outer HCs positioned more laterally within the organ of Corti (Fig 1). Supporting cells, including Hensen's cells, Deiters' cells, pillar cells and phalangeal cells, intermingle with HCs in the cochlea. HCs are susceptible to degradation with age and can sustain damage from loud noises, and treatments like those used in infections or cancer chemotherapy. In mammals, lost HCs cannot be repaired or replaced. While extensively studied in mice, studying HCs poses challenges due to their limit-

* Corresponding author: Somayeh Niknazar, niknazar.somayeh@gmail.com, niknazar@sbmu.ac.ir
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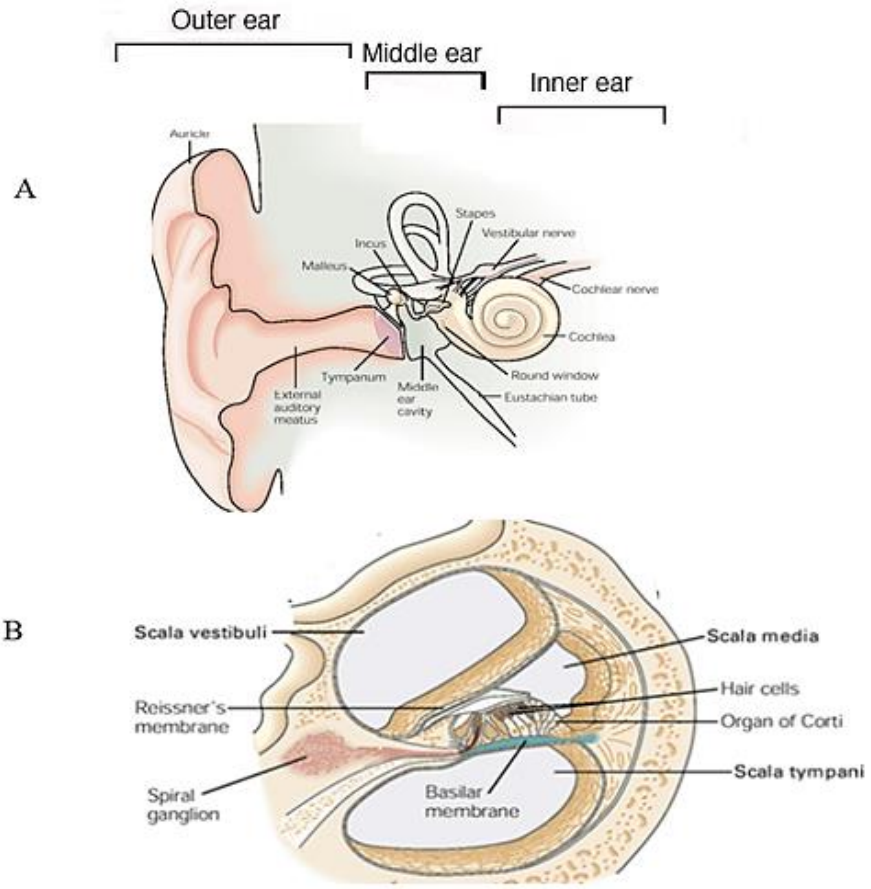

FIGURE 1. The anatomy of the human ear and cochlear partition. (A) The human ear consists of three primary components: the outer, middle, and inner ear. (B) In the cochlea, a cross-sectional view reveals three ducts: the scala vestibuli, scala media (or cochlear duct), and the scala tympani. Scala media, an endolymph-filled compartment includes sensory hair cells (Adapted from Noback 1967). (Noback, 1967; Kandel et al., 2000).

ed numbers in the inner ear and their deep location within the temporal bone. Researchers are looking for ways to grow HCs in the laboratory to better understand their functionalities and the contributing factors to their degradation and loss (Menendez et al., 2020). Several experimental studies have shown the regeneration of auditory neurons through the application of exogenous neurotrophins in cases of sensorineural hearing loss (Gillespie and Shepherd, 2005; Shepherd et al., 2008; Bader et al., 2010; Chen, 2011; Ortiz-Marquez et al., 2013). However, the neuroprotective effect of neurotrophic factors was not observed after treatment (Momeni et al., 2011). Long-term delivery of exogenous neurotrophins to the cochlea using pump-based systems presents challenges due to increased infection risks associated with device reloading or replacement, commonly applied in laboratory animal studies (Chuang, 2012). In regarding to these difficulties, researchers are developing multiple strategies to direct the differentiation of progenitor and exogenous cells towards the regeneration of functional HCs and auditory neurons (Chen et al., 2012; Okano and Kelley, 2012; Peyvandi et al., 2018a; Peyvandi et al., 2018b). Cell therapy holds promise as a treatment for sensorineural hearing loss, potentially enabling the repair or replacement of lost HCs. Utilizing various sources of adult and embryonic stem cells in monoculture has been investigated for repairing or replacing injured HCs (Zengler et al., 2002). Interactions and signal transduction between developing tissues play important roles in the regulation of differentiation in vivo. Due to cellular signaling pathways complexity, it is often difficult to completely mimic this environment in vitro (Peyvandi et al., 2018a; Peyvandi et al., 2018b). Efforts have been made to derive auditory neurons from stem cells established *in vitro* or through co-culture approaches, aiming to repair or regenerate hearing loss caused by sensorineural issues (Coleman et al., 2007; Sambandam, 2018). The purpose of this study is to review the

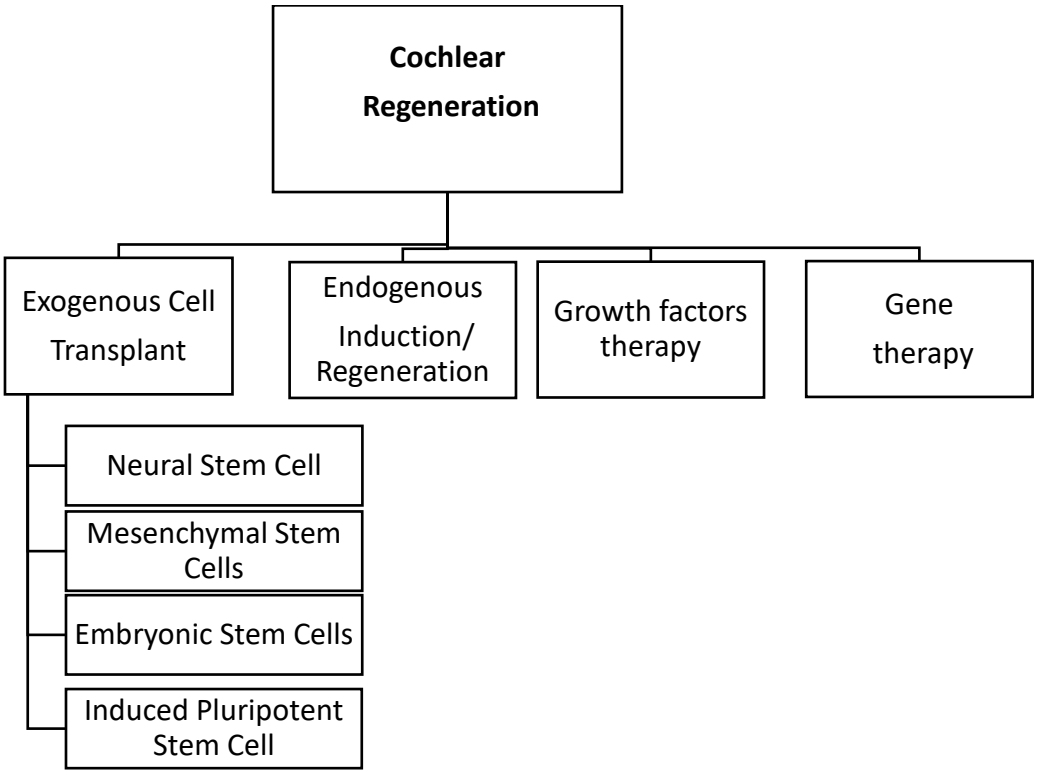

FIGURE 2. Biological approaches for cochlear regeneration in sensorineural hearing loss

induction procedures for stem cell differentiation into cochlear HCs or auditory neurons, offering a potential therapeutic approach for treating deafness.

Literature Search

Papers were collected from Web of Science, PubMed, Scopus, and Google Scholar electronic databases from 2000 to 2022 to discuss the development of cell therapy approaches for damaged cochlear HCs regeneration or replacement which help in resolving deafness. Also, an e-book (1967) was used for schematic illustrations.

Conventional Approaches

Repairing HCs damage is important for maintaining auditory function throughout life, as mammalian HCs do not replace or regenerate. For decades, researchers across the globe have been dedicated to discovering cures for deafness (Nakagawa, 2014). Although cochlear implants have gained popularity for managing hearing loss, they do not cure deafness or restore natural hearing. These devices transform input sounds into electrical stimuli within the Spiral ganglion neurons (Sprinzl and Riechelmann, 2010). Recent biological approaches aiming to regenerate cochlear HCs in mammals offer promising directions for treating deafness by modulating molecular pathways or through cell transplantation (Steel and Kros, 2001; Li et al., 2004) (Fig 2).

Cochlear Implant

A cochlear implant (CI) is a small electronic device designed to electrically stimulate the SGNs within the cochlea of deaf people, enabling them to receive and interpret sound. By bypassing the normal auditory transmission pathway through hair cells, a CI provides direct electrical stimulation to the SGNs. In fact, it bypasses the damaged part of the cochlea and directly stimulates the remaining auditory neurons. While cochlear implants do not cure or restore hearing, they help people with severe or complete deafness in perceiving sound (Ramsden, 2002).

Growth Factors Therapy

Growth factors, a class of secretory proteins, play critical roles in cell survival, proliferation, and differentiation, particularly in various conditions aimed at differentiating auditory HCs (Bakhtiarzadeh et al., 2018; Mahmoudian-Sani et al., 2018). Three families of growth factors, including fibroblast growth factor

(FGF), neurotrophin (NT), and insulin growth factor-1 (IGF-1), are involved in otic neurogenesis. In this regard, auditory neurons can connect to the sensory mechanoreceptors, HCs, and central nervous system of the ear (Alsina et al., 2003). Neurotrophic factors are critical for the developing auditory system and innervation during development. In addition, they are necessary for the survival and maintenance of SGNs in adulthood. Treatment with neurotrophins protects and prevents cochlear cells from degeneration caused by drugs, noise, or aging (Niknazar et al., 2022) (Mahmoudian-Sani et al., 2018). Simultaneous application of multiple growth factors with neomycin has significant effects in reducing HC loss. Among these, epidermal growth factor (EGF) has proven effective in supporting the survival of outer HCs (Lou et al., 2015). EGF receptor transcripts are upregulated in neomycin-treated cochlear epithelium in 3-day-old rats, that increases mammalian neonatal HC replacement process following neomycin induced HC toxicity (Zine et al., 2000). Furthermore, interventions involving brain derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF) have enhanced the survival and function of SGNs in guinea pig models of hearing loss (Shinohara et al., 2002). Studies administrating glial-derived neurotrophic factor (GDNF) to the inner ear of hearing-impaired animals have reported protective effects on SGNs survival and electrical responsiveness (Maruyama et al., 2008; Fransson et al., 2010). Current challenges associated with neurotrophic factors therapy is how to deliver them to the cochlea. Several methods such as osmotic mini-pumps, carrier-based delivery, or encapsulated cell targeted delivery have been applied. In addition, the long-term effects of exogenous neurotrophin delivery pose challenges due to a heightened risk of infection (Shinohara et al., 2002; Agterberg et al., 2009; Pettingill et al., 2011).

Totally, growth factors are important factors during the development of the auditory system and ganglion cell innervation. They are also essential for maintaining adult SGNs, protecting neuronal populations from injury, or stimulating neuronal regeneration and repair after injury.

Gene Therapy

Gene therapy, utilizing exogenous DNA delivery or genome editing agents to the cochlea, is another option to treat hearing disorders. So far, numerous vectors from different virus families such as adenovirus, adeno-associated virus or lentivirus have been tested. Protective effects of adenovirus-mediated overexpression of GDNF against gentamicin ototoxicity have been shown by Suzuki et al (Suzuki et al., 2000). Nonviral vector systems, including plasmids and lipid-covered packages are also alternatives methods for gene delivery. As well, electroporation technology has been developed to manipulate gene expression in postnatal rat cochlear explants using the pCligGFPAtoh1 vector (Zheng and Gao, 2000). In addition, CRISPR-associated protein 9 (CRISPR/Cas9) has greatly enhanced genome editing efficiency in modifying mutant alleles for treating hearing loss in animal models. Currently, the combination of iPSC modeling and CRISPR / Cas9 gene editing shows great potential for gene and stem cell therapies in hearing loss tratment (Nourbakhsh et al., 2021).

The closed space of the inner ear makes gene or drug delivery highly efficient compared to systemic delivery, where large doses are often required to reach the inner ear. Recent advancements in inner ear gene therapy show increased accessibility, offering promising avenues for further research. Continuous efforts to improve cochlear gene delivery methods hold the potential to establish gene therapy as a viable treatment for hearing loss.

Cell Therapy

Stem cells in several mammalian tissues has the potential to self-regenerate and aid in the repair of injured tissue. Investigations into stem cell therapy for addressing hearing loss primarily concentrate on their potential to develop and function as HCs. Researchers are developing multiple strategies to guide exogenous and progenitor cells towards the regeneration of functional HCs or auditory neurons within the inner ear (Niemeyer et al., 2011; Goers et al., 2014; Krinner and Roeder, 2014), (Table 1).

Strategies to Induce Otic Cell types From Stem CellsEndogenous Stem Cells

HCs in the inner ears of non-mammalian vertebrates, including birds, amphibians, and fish, exhibit a lifelong ability to regenerate (Warchol et al., 1993; Goers et al., 2014). In contrast, the adult mammalian auditory system fails to regenerate damaged hair cells, resulting in permanent hearing loss. Recent studies, however, have demonstrated the differentiation and regeneration of

TABLE1: The effect of exogenous stem cells in the treatment of hearing impairment in animal models. Neural stem cells (NSCs), Bone marrow mesenchymal stem cells (BM-MSCs), Rosenthal's canal (RC), Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE). Spiral ganglion neurons(SGNs), Auditory nerve (AN), Human otic progenitor cells (hOPCs).

Type of Stem cells		**Model**	Transplantation Delivery Approach	Target	Outcome of Cell therapy	References
Neural Stem Cells	Rat Hippocampus-derived NSCs	Rat Model of Sensorineural Hearing Loss	Cochlear wall or round or oval windows	Cochlear tissue	Transplanted NSCs were integrated into the organ of Corti and some HSCs developed into hair cells.	(Ito et al., 2001)
	Mouse NSCs	Guinea pig model of neomycin induced hearing loss	Cochleostomy	Cochlear neurons or sensory epithelia	Transplanted NSCs survive and differentiate in the injured inner ear	(Hu et al., 2005)
	Rat olfactory epithelium NSCs (oe-NSCs)	Rat Model of noise-induced hearing loss	Retroauricular	SGNs in Rosenthal's canal	Restoration of hearing function (ABR). Implanted oe-NSCs survived and migrated into RC around SGNs.	(Xu et al., 2016)
	Mouse NSCs	Cisplatin-treated cochleae of mice	Round window	SGNs	Grafted NSCs differentiate into neurons in the modiolus and restore spiral ganglion neurons	(Tamura et al., 2004)
	Mouse NSCs	Sound damaged cochleae of mice and guinea pigs	Round window	Cochlear tissue	NSCs integrated into the cochlear tissue and differentiated into a several of cochlear cell types	(Parker et al., 2007)
Mesenchmal Stem Cells	Human BM-MSCs	Rat model of noise or ototoxic drug induced hearing loss	Intravenous injection	Spiral ganglion area	Migration of cells into the cochlea was associated with the BDNF expression	(Choi et al., 2012)
	Rat BM-MSCs	Rat model of Kamamicin induced hearing loss	Transtympanic delivery	Cochlear tissue	Hearing normal function (ABR and DPOAE)	(Mittal et al., 2020)
	Rat BM- MSCs	Rat Model of Acute Sensorineural Hearing Loss	lateral semicircular canal	Cochlear fibrocytes	Improved hearing recovery (ABR) and repaired interrupted gap junction network	(Kamiya et al., 2007)
	Mice BM- MSCs	C57BL/6J Mice	Posterior semicircular canal	Cochlear fibrocytes	Differentiation of transplanted MSCs into fibrocyte-like cells without adverse side effects on hearing function	(Kasagi et al., 2013)
	Human Placenta- MSCs	Guinea pig Model of Acute Sensorineural Hearing Loss	Brachial vein	SGNs	Increase in number of Spiral ganglion neurons and improvement of auditory function (ABR and DPOAE)	(Kil et al., 2016)
	Human ADSCs	Rat Model Of β-tubulin induced autoimmune sensorineural hearing loss	Intraperitoneal	Cochlear Hair cells	improved hearing function (ABR) and protected hair cells	(Yoo et al., 2015)

Type of Stem cells		Model	Transplantation Delivery Approach	Target	Outcome of Cell therapy	References
Induced Pluripotent Stem Cell	Mouse IPSCs	Slc26a4-null mice	Round window	Cochlear Hair cells	The transplanted OEPs derived from iPSCs differentiated into hair cells. The dendritic endings of native SGNs formed synaptic connections with the adjacent regenerated hair cells.	(Chen et al., 2018)
	Human IPSCs	Guinea pig model of Amikacin induced sensorineural hearing loss	Cochleostomy	Cochlear Tissue	The iPSC-derived-hOPCs were incorporated into the ototoxin-exposed cochlear sensory epithelium, and partially differentiated into cells expressing initial HC and supporting cell markers.	(Lopez-Juarez et al., 2019)
	Human IPSCs	Intact guinea-pig cochleae	Cochleostomy	SGNs	Human iPSC-derived neurons contributed to the settlement of transplant-derived neurons expressing VGLUT1 in guinea-pig cochleae	(Ishikawa et al., 2017)
	Mouse IPSCs	Wistar albino rats with hearing impairment	Cochleostomy	Cochlear Tissue	Transplanted iPSCs did not show a therapeutic effect	(Gökcan et al., 2016)
Embryonic Stem Cells	Mouse ESCs	Mongolian gerbils with hearing loss (Ouabain)	Round window	SGNs	Small number of transplanted ESCs differentiate into mature neuron- and glia-like cells	(Lang et al., 2008)
	Mouse ESCs	Deafened guinea pig (Kanamycin)	Round window	Auditory primary neurons, Spiral ganglion neurons.	functional restoration of spiral ganglion neurons (ABR)	(Okano et al., 2005)
	tau-GFP mouse ESCs	Deafened Sprague–Dawley rats (β-bungarotoxin)	Scala tympani	AN	Cells were detected in scala tympani, modiolus, AN trunk and the brain stem.	(Palmgren et al., 2012)
	mouse ESCs	Deafened guinea pig	Round window	SGNs	A small number of ESCs survived in the deafened cochlea for up to 4 weeks, without eliciting an inflammatory response.	(Coleman et al., 2006)

HCs from endogenous inner ear stem cells. During this regeneration process, endogenous stem cells can differentiate into HCs through a combination of mitotic and non-mitotic mechanisms, triggered by signals sent by dying hair cells (Li et al., 2003a; Oshima et al., 2007).

Research has also revealed the innate ability of adult mammalian vestibular HCs to self-repair following injury. Notably, apart from the vestibular system, the cochlea of newborn mammals also contains endogenous stem cells (Kelley, 2006; Brigande and Heller, 2009). Therefore, a promising approach to treat SNHL involves either activating endogenous stem cells or inducing genetic modifications in the remaining hair cells. Recent studies have shown that inner ear stem/progenitor cells can be isolated from the neonatal cochlea (Oshima et al., 2007). Moreover, the overexpression of several genes in adult cochlear cells suggests an inherent capacity for repair when stimulated by specific signals (Izumikawa et al., 2005).

The presence of stem cell inside the cochlea raises the hope for the regeneration of inner ear cells. However, the loss of these stem cells postnatally correlates with a decline in regenerative potential and can restrict our ability to stimulate regeneration. Hence, future strategies for regeneration should carefully consider the distribution of endogenous stem cells in the inner ear and whether cells retaining regenerative potential are conserved.

Exogenous Stem Cells

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are adult cells identified by their non-hematopoietic nature, multipotency, high self-renewal capacity, rapid proliferation rate, differentiation potential, paracrine activity, and ability to migrate to injury sites (Guadix et al., 2017). MSCs derived from sources like bone marrow, blood, adipose tissue, umbilical cord, and placenta which applied in various tissue regeneration studies (Ding et al., 2011). Among these, bone marrow-derived MSCs hold promise for inner ear cell replacement therapy. The differentiation of MSCs into auditory HC –like cells and neuronal cells needs factors such as BDNF, GDNF, neurotrophin-3 (NT-3), and growth factors (Mahmoudian-Sani et al., 2017; Gonmanee et al., 2018; Young et al., 2018). In a study by Sang-Jun Jeon, MSCs obtained from mouse bone marrow were differentiated into sensory HCs. MSCs were first transfected with the transcription factor EGFP-Math1 and then differentiated into sensory progenitor cells through a two-week culture involvingIGF1, EGF, bFGF, NT3, and BDNF. Overexpression of Math1, a key HC development inducer, augmented the expression of HC markers in the sensory epithelium. Co-culture with chick otocyst cells further increased the expression of HC-related markers such as myosin VIIa, p27Kip, Brn3c, jagged2, and Math1, suggesting MSCs' differentiation to inner ear sensory cells (Jeon et al., 2007). It was reported that MSCs derived from rat bone marrow could differentiate into HCs in culture. MSCs were first differentiated into neuron-like cells. Neural differentiation involved culturing BMSCs (at passage 4) in DMEM/F12 supplemented with B27, bFGF, and EGF.For the HC –like cell induction, neural stem cells were cultivated in a serum-free medium comprising DMEM/F12, B27, bFGF (Basic fibroblast growth factor), EGF, and IGF-1 for 14 days, resulting in the detection of HC markers like Myosin VIIA via immunocytochemistry (ICC) assay (Qin et al., 2011; Niknazar et al., 2019MSCs-based therapies have been widely used in the treatment of various disorders (Wu et al., 2014). Systemic administration of MSCs in noise or drug-induced cochlear damage models triggers abundant neurotrophin release at the injured cochlea site, enhancing the regeneration of auditory HCs and neurons (Choi et al., 2012). In the sensorineural hearing loss model, MSCs transplantation has led to functional hearing recovery by repairing damaged spiral ligament fibroblasts (SLF) and damaged gap junction networks in the cochlear lateral wall, facilitated by the migration and proliferation of MSCs (Kamiya et al., 2007). In young mice cochlea , implanted MSCs migrated and transformed into fibroblast-like cells without affecting auditory function, suggesting that MSCs transplantation could potentially slow or halt the early progression of sensorineural hearing loss (Kasagi et al., 2013).

The application of cell therapy utilizing MSCs for damaged cochlear tisshues holds promise for regenerating HCs, SGNs, and spiral ligaments. Indeed, MSCs therapy contribute to slowing the progression of hearө ing loss.

Neural Stem Cells

Neural stem cells (NSCs) can regenerate themselves and differentiate into various cell types including neurons, oligodendrocytes, and astrocytes (Clarke et al.,

2000; Gage, 2000). Several studies have shown that the use of NSCs in the treatment of SNHL is promising by replacing spiral ganglion neurons or HCs within the inner ear NSCs were transduced with neurogenin 2 (ngn2) before transplantation into both normal and hearing-loss animal cochlea, resulting in notably low survival rates of transplanted cells in the ngn2-introduced group (Hu et al., 2005). Another study involved transplantation of adult mouse NSCs into the inner ears of mature guinea pigs to investigate their potential for survival and differentiation into auditory neurons (Carricondo and Romero-Gómez, 2019). Parker et al., revealed that transplanted NSCs in acoustically damaged cochlea migrated throughout the cochlea, expressing markers for HCs and SGNs (Parker et al., 2007). A recent study investigated the therapeutic effect of olfactory epithelium neural stem cells (oeNSC) on noise-induced hearing loss in a rat model. Transplanted oeNSC successfully ameliorated deafness in rats, evidenced by improved auditory brainstem response (ABR) results. SGNs incubation with oe-NSCs led to enhanced release of neurotrophic factors such as NGF and NT-3, indicating the potential of oe-NSCs in upregulating neurotrophic factor expression and thus aiding in recovery (Xu et al., 2016).

Multiple investigations have highlighted the promise of NSCs in SNHL treatment by either replacing damaged inner ear cells or aiding in their repair and regeneration. NSCs exhibit the potential to differentiate into hair cells, supporting cells, and auditory neurons within the inner ear, offering hope for future therapeutic interventions.

Embryonic Stem Cells

Previous study has shown that the differentiation of the otic progenitor cells is independent of external signals from neighboring cells for suitable differentiation (Oshima et al., 2010). In vitro differentiation of human embryonic stem cells into either HCs or sensory neurons is associated with the expression of several well-characterized markers at each stage. However, auditory neuron and sensory cell culturing revealed grand challenges in terms of both culture media and the necessary differentiation factors for alteration of progenitor cells into the specified cell type (Dufner-Almeida et al., 2019). Stem cell-derived murine progenitor cell, whether post-transplant in chicken embryonic ears, within 3D aggregates, or within the presumptive otic progenitor cells, needed co-culture with mesenchymal stromal cells from the chicken utricle to achieve proper cell morphological maturation (Li et al., 2003b; Koehler et al., 2013). Serum-free media containing supplements like N2 and B27 have shown potential in inducing neural progenitor cells expressing specific markers of ear placode neuroblasts such as Otx2, NeuroD, Brn3a, Nestin, Pax2, and Pax8 as a starting point for ESC differentiation (Chen et al., 2012) (Shi et al., 2007; Ronaghi et al., 2014). Studies investigating ESCs for the replacement of HCs and SGNsin inner ear regeneration have utilized various approaches.. In early studies, mouse ESCs labeled with green fluorescent protein (GFP) were introduced into the cochlea (Hildebrand et al., 2005). Co-culture of human ESCs with mouse cochlear sensory epithelium in the presence of bone morphogenetic protein 4 (BMP4) was found to be crucial for inducing the expression of markers like GATA3, Peripherin, Tropomyosin receptor kinase B (TrkB), TRKC and ngn1, markers of auditory neurons, in some cells (Ronaghi et al., 2014). However, only a small fraction of these cells expressed three more HCrelated markers, including MYO7A, MYO15A, ATOH1, and OTOF (Otoferlin). Progenitor cells derived from human ESCs, when differentiated, have demonstrated the ability to mature in mouse organ of corti explants, expressing tubulin and synapsin at the junction field. Sixty days after transplantation of neural progenitor cells that previously differentiated from human ESCs into the base of a gerbil- deafferented cochlea, abundant new neurites protruding towards the apex of the cochlear nerve trunk were detected (Shi et al., 2007).

The potential of ESCs to generate multiple cell types has prompted studies exploring their use in replacing HCs and SGNs for inner ear regeneration. Additionally, being derived from the patient's own cells, ESCs are considered autologous and do not pose the risk of immune rejection.

Induced Pluripotent Stem Cells

Pluripotent stem cells (PSCs) have the ability to remain undifferentiated, self-replicate and differentiate over extended periods (Thomson et al., 1998 thesis). Induced pluripotent stem cells (iPSCs) are shaped through the compelled expression of numerous transgenes, generally a combination of Sox2, c-Myc, Oct3/4, and Klf4, which reprogram somatic cell nuclei from human,

monkey, rat, mouse, and canine origins (Nelson et al., 2010). Both hESCs and iPSCs are considered pluripotent. Human iPSCs have been considered as an effective alternative for human ESCs. Thus, the ability of these cells makes them as a viable option for cell-based therapies (Yu and Thomson, 2014). In a study by Oshima et al., HC-like cells derived from murine ES and iPS cells responded to mechanical stimuli. The differentiation of HC-like cells showed no significant difference between ESCs and iPSCs. Both murine ESCs and iPSCs demonstrated the ability to generate otic progenitor cells capable of differentiating into mechanosensory HCs in vitro. These cells expressed specific markers for HCs and responded to mechanical stimulation, similar to the transduction currents of immature HCs (Oshima et al., 2010).

Gunewardene et al., successfully established a neurosensory cell line in vitro using two human foreskin-derived iPSC lines. The step-by-step differentiation process yielded electrophysiologically active sensory neurons, displaying activity patterns resembling those of auditory neurons in early postnatal mice (Gunewardene et al., 2014). In the work by Oshima et al., iPSCs derived from Atonal homolog 1 (Atoh1) / nGFP transgenic mouse fibroblasts were cultured with Smad3 inhibitors, Wnt pathway inhibitors, and IGF1 for five days, followed by exposure to bFGF for three days to induce differentiation into ear progenitor cells (Oshima et al., 2010). These cells then differentiated into HCs by co-culture with mitotically inactivated embryonic chicken utricle stromal cells. However, the overall efficiency of HC differentiation from iPSCs was low, with approximately about 12% of iPSC-derived HCs expressing Myosin VIIA (MYO VIIA), while only 2.6% expressing both MYO VIIA and ESPIN. These iPSC-derived HCs exhibited morphological and electrophysiological characteristics of immature HCs.

Chen et al., also succeeded in obtaining HCs from human iPSCs (Chen et al., 2018). They utilized iPSCs derived from human urinary cells, subjecting them to a HC differentiation protocol similar to ESCs, resulting in the production of HC-like cells with an efficiency of up to 50% (Chen et al., 2012). Another study investigated the effect of mouse iPSCs in a SNHL model by differentiating them into HCs and SGNs within the mouse cochlea. CM-Di1-positive iPSCs were detected in the modiolus and Rosenthal's canal of the cochlea 4 weeks after implantation. Some of these cells expressed markers of HCs or SGNs. While the transplantation of iPSCs slightly improved the ABR threshold, there was no significant difference between pre- and post-transplant iPSCs. Notably, the transplanted iPSCs were able to migrate and transform into HC-like and SGNs-like cells within the cochlea (Nishimura et al., 2009).

Overall, iPSCs have demonstrated promise in regenerating inner ear cells across various studies (Dufner-Almeida et al., 2019). However, further research is needed to enhance the survival and differentiation of iPSCs in the cochlea for more effective application in hearing loss therapies.

Clinical Application of Stem Cells for Inner ear

The clinical application of stem cells for hearing loss treatment is still in its early stages. Studies examining the transplantation of autologous BM-MSC in patients with SNHL did not yield clinically significant improvements in hearing. However, a 3-year follow-up showed no complications or side effects (Lee et al., 2018). Another study by Baumgartner et al., investigated the effect of autologous umbilical cord stem cells (HUCB) in acquired SNHL in children. The clinical trial involved audiological assessments such as distortion product otoacoustic emissions (OAE), ABR, tympanometry, audiogram, and brain MRI (magnetic resonance imaging) before and after a single intravenous administration of HUCB. Encouragingly, no observed toxicity or complications were reported. Children who received a higher dose of cells exhibited a decline in ABR threshold, alongside increased white matter areas in the primary auditory cortex observed in fractional anisotropy of MRI (Baumgartner et al., 2018).

In addition, current gene therapy research has focused on treating genetic deafness using patient- iPSCs (Nourbakhsh et al., 2021). Another study showed that hiPSC lines reprogrammed using non-integrated mRNA expressed ear markers PAX8, PAX2, FOXG1, and SOX2, and subsequently differentiated into hair cell and neuronal lines. This research highlights that mRNA-reprogrammed iPSCs can generate ear lineages similar to those induced by lentivirus, while being safer for potential clinical applications (Boddy et al., 2020).

Presently, there are no Food and Drug Administration (FDA)- approved treatments for cell therapy usage in hearing loss. However, ongoing clinical trials aim to determine the safety and efficacy of cell therapy in treating

hearing loss in humans.

Conclusion

While both endogenous and exogenous stem cell applications have shown promise in various animal models, their translation to human treatments is hindered by several limitations. Addressing these limitations is imperative before considering the implementation of these treatment options in clinical settings. Thus, future studies should prioritize assessing the functional integration of engrafted cells into the intricate hearing system of animal models. Presently, targeted differentiation of stem cells into HCs or neurons, exploration of optimal methods for stem cell transplantation into the inner ear, and rigorous safety evaluations constitute the groundwork for advancing towards clinical cell therapy.

In addressing sensorineural hearing loss (SNHL), conventional treatments such as hearing aids and cochlear implants have been pivotal in improving patients' hearing capabilities However, the efficacy of these interventions depends on the remaining HCs and spiral neurons. Therefore, current research in audiology emphasizes the development of several approaches aimed at initiating HC regeneration or replacement within the inner ear thereby offering potential solutions for deafness. Although both endogenous and exogenous applications of stem cells have shown promise in several animal models, there are numerous limitations that need addressing before implementing these treatment options in humans. Therefore, further studies should focus on the efficient functional integration of engrafted cells into the hearing systems of animal models. Currently, targeted differentiation of stem cells into HCs or neurons, along with research on methods for stem cell transplantation into the inner ear and their safety, lay the foundation for clinical cell therapy.

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Conflict of Interests

There are no conflicts of interest to declare.

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