ISSN: 2083-389X
eISSN: 2084-3127

J Hear Sci, 2024; 14(3): 19–36
DOI: 10.17430/jhs/191685

COCHLEAR HAIR CELL REGENERATION BASED ON STEM CELLS: A SYSTEMATIC REVIEW

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Contributions:
A Study design/planning
B Data collection/entry
C Data analysis/statistics
D Data interpretation
E Preparation of manuscript
F Literature analysis/search
G Funds collection

Abstract

Introduction: According to the World Health Organization (WHO), by 2050 at least 700 million people will need access to hearing care and hearing rehabilitation services. The search for cell or gene therapies has been intensifying, and stem cell therapy looks a promising candidate to support hearing regeneration and reduce these numbers. The aim of this study is to provide an overview of current advances in stem cell-based therapies for cochlear hair cell regeneration and the processes being developed for future applicability.

Material and methods: Identification and review of all articles in the databases PubMed, Web of Science, and PLoS One using the terms *stem cell*, *auditory hair cell regeneration*, and *mammalian* during February 2023 and following the PRISMA guidelines.

Results: 50 articles were obtained, published between 2003 and 2022 and were systematically analyzed. The current research quantity is limited and further studies are needed, particularly in human tissue.

Conclusion: The simultaneous use of cell therapy and gene therapy may lead to more promising results. Moreover, advances in cochlear hair cell regeneration with stem cells suggest there is a realistic potential to make the technique a useful future therapy.

Keywords: transplantation • stem cell • regeneration • inner ear • hair cell

REGENERACJA KOMÓREK SŁUCHOWYCH OPARTA NA KOMÓRKACH MACIERZYSTYCH: PRZEGLĄD SYSTEMATYCZNY

Streszczenie

Wprowadzenie: Według Światowej Organizacji Zdrowia (WHO) do 2050 roku co najmniej 700 milionów ludzi będzie potrzebowało dostępu usług w zakresie protetyki słuchu i rehabilitacji słuchu. Z tego względu intensyfikowane są poszukiwania odpowiednich terapii komórkowych lub genowych, a terapia komórkami macierzystymi celem wspomagania regeneracji słuchu i zmniejszenia liczby potrzebujących wydaje się obiecująca. Celem niniejszego badania jest przedstawienie przeglądu aktualnych wyników terapii opartych na komórkach macierzystych w regeneracji komórek słuchowych oraz procesów opracowywanych pod kątem przyszłego zastosowania.

Materiał i metoda: Dokonanie przeglądu wszystkich artykułów wyszukanych w bazach PubMed, Web of Science i PLoS One w lutym 2023 roku przy użyciu terminów *komórka macierzysta*, *regeneracja komórek słuchowych ślimaka* i *ssaki* oraz zgodnie z wytycznymi PRISMA.

Wyniki: Uzyskano 50 artykułów opublikowanych w latach 2003–2022 i poddano je analizie systematycznej. Obecna liczba badań jest ograniczona i potrzebne są dalsze badania, szczególnie na tkankach ludzkich.

Wnioski: Jednoczesne stosowanie terapii komórkowej i terapii genowej może prowadzić do uzyskania bardziej obiecujących wyników. Co więcej, postępy w regeneracji komórek słuchowych za pomocą komórek macierzystych sugerują, że istnieje realny potencjał, aby uczynić tę terapię użyteczną w przyszłości.

Słowa kluczowe: transplantacja • komórka macierzysta • regeneracja • ucho wewnętrzne • komórka słuchowa

Key for abbreviations

cHCs	cochlear hair cells
CIs	cochlear implants
HCs	hair cells
IHCs	inner hair cells
OHCs	outer hair cells
SC	stem cell
vHC	vestibular hair cell
WHO	World Health Organization

Introduction

The cochlea is responsible for the processing of sound in the initial phase of the auditory pathway; it is a sound transducing organ capable of transforming the hydro/biomechanical energy coming from the middle ear through the oval window into electrophysiological energy.

In the organ of Corti there are supporting epithelial cells and specialized sensory cells called cochlear hair cells (cHCs). The cHCs of the inner ear are mechanoreceptors that transform acoustic signals into electrochemical signals through the displacement of stereocilia [1]. There are two groups of cHCs: the inner hair cells (IHCs) and the outer hair cells (OHCs). The OHCs (12,500 of them) are much more plentiful than IHCs. OHCs have long, thin stereocilia; they form later in embryonic development, and are more easily damaged than IHCs. IHCs are less numerous (3500 of them); they develop earlier and are more resilient [2].

Most cases of hearing impairment are due to the degeneration of cHCs. Damage to these cells is mainly induced by age, anoxia at birth, infection, exposure to ototoxic drugs (e.g. antibiotics, some anti-cancer drugs), genetic mutations, and exposure to high-intensity sounds. Hearing deficits may also result from damage to the neurons of the spiral ganglion that innervate the cHCs [3]. Regeneration of cHCs after damage occurs spontaneously in non-mammalian vertebrates like birds and fish but not in the mammalian cochlea, meaning that in mammals hearing loss is permanent [4].

Therapeutically, a range of hearing support technologies exist, such as hearing aids and implantable medical devices. However, in aiming to restore hearing, remarkable advances have led to other innovative therapies [5]. For cochlear implants (CIs) to be successful and effective, afferent neurons must be functional [6]; if they are not, CIs may be contraindicated, even if conventional hearing aids fail to provide any benefit. The search for new solutions has led to stem-cell therapy (SC) [7]. Continued research into the regeneration of cHCs suggests that future treatment of sensorineural hearing loss may involve a combination of gene therapy, cell therapy, molecular therapy, and CIs [8].

It is now possible to convert differentiated somatic cells into multipotent SCs that have the capacity to generate all adult cell types; this technique is called induced pluripotent stem cells. Thus, there is a wide variety of applications for this technology, including regenerative medicine, *in vitro* disease modeling, and drug screening/discovery [9].

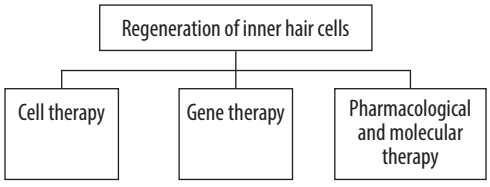

Figure 1. There are three main approaches to stem cell therapy: cell therapy, gene therapy, and pharmacological and molecular therapy

According to Diensthuber and Stöver, and as shown in **Figure 1**, cochlear or inner ear hair cell regeneration encompasses cell therapy, gene therapy, and pharmacological and molecular therapy [2,10].

Stem cell-based hair cell regeneration

The inability of mammals to regenerate their hearing organ after damage is due to the postnatal decrease in SCs in the inner ear [11,12]. Generally speaking, there are two models for studying hair cell regeneration in mammals, namely cochlear organoids and cochlear organs. Hair cells within the organoids derived from pluripotent stem cells, or from a cochlear progenitor, share similar structural and functional properties to native hair cells. The inner ear and cochlear organoids can be derived from induced pluripotent SCs [13]. Induced pluripotent SCs are generated via genetic reprogramming of adult somatic cells that have limited differentiation potential but, upon reprogramming, express genes that enable them to regain plasticity and give rise to all cell types [14–16].

Previous studies have described SC therapy in which cells are transplanted into the inner ear to replace injured or dead cHCs [17–19]. To develop successful regenerative approaches for hearing loss, there must be a detailed understanding of the human inner ear, specifically its function, differentiation, and cellular mechanisms. There are currently several ongoing early-stage studies into the regeneration of cHCs.

Techniques that use SCs as a basis for cHC regeneration could play a key role in hearing restoration [5,10]. There are a large number of possible sources for obtaining SCs for hearing therapy, including: embryonic SCs, induced pluripotent SCs, mesenchymal SCs, neural SCs, and inner ear SCs. It is concluded that SC-based therapy looks especially promising for re-establishing hearing function [20–22]. There are two possible SC-based approaches to treating deafness [3,21–23]:

– endogenous regeneration or the restoration of existing HCs in the inner ear by inducing changes at the cell cycle level (administration of cell survival factors and other biologically active molecules), stimulating resident SCs within the organ of Corti to replace their own damaged cHCs;

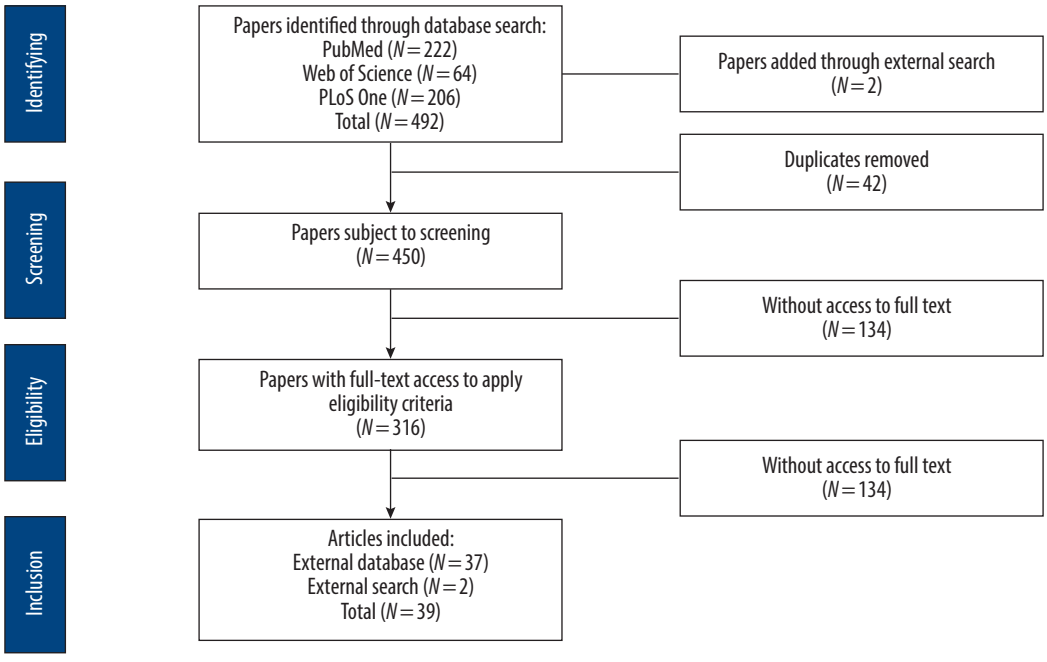

Figure 2. PRISMA flow chart

– exogenous delivery of SC, i.e., introduction/transplantation of SC into the inner ear.

The procedures/treatments required for endogenous activation of the inner ear in humans still remain unknown (at present); however, it is felt that SC transplantation has a higher chance of success compared to endogenous regeneration [22,23]. In order to repair the auditory sensory epithelium, identification of specific and appropriate cellular markers of inner ear SCs is needed so that endogenous inner ear SCs can be differentiated from exogenous SCs [23–25].

Cochlear support cells, once cultured and isolated *in vitro*, have the ability to express markers of the cHC, inferring that they can differentiate into this cell type. These support cells, unlike SCs, do not self-renew, so researchers have questioned if they are the best target, or if there are other undifferentiated cochlear cells with better chances of creating cHCs [26,27]. For successful regeneration of cHCs, the replacement of neural fibers is also important [28].

Practical challenges to SC transplantation

The transplantation of SCs into the ear is complex. The literature records that there are several anatomical structures on the way to the final target site, highlighting the tympanic scala, scala media, and Rosenthal's canal [3,23], as well as the vestibular scala [11,29,30], the lateral cochlear wall [28,29,31], the perilymphatic/modiolus perforation [32,33], the round window [34,35], the lateral semicircular canal [36], and the auditory nerve [3].

Where do we stand regarding advances in SC-based therapies for ear regeneration? What processes are being developed for future applicability and what will be the impact in audiology clinical practice? These are questions that this review addresses.

Material and methods

A systematic literature review on the topic of *Stem cell-based regeneration of cochlear hair cells* was carried out to see what processes are being tested and how the research is progressing. Articles were searched in PubMed, Web of Science, and PLoS One, using the Boolean operator AND with the terms "stem cells, auditory hair cells, regeneration, and mammalian." Other articles were also searched through external academic libraries, and 2 articles considered relevant from this external search were also included in the review.

The inclusion criteria included articles published in English, Portuguese, or Spanish, with full-text access, applicability to humans, and mentioning the term stem cell(s) in the title or abstract.

A search in February 2023 found 492 articles. Duplicates were removed and complete articles without full text online access were requested by mail direct from the first authors, leaving a selection of full text articles. Data extracted from each full text article for eligibility assessment included: Authors; Year of publication; Country; Type of therapy; Types of regenerated hair cells; Population (species); Anatomical route of cochlear transplantation; Stem-cell classification; and Findings and suggestions. The complete process is shown in **Figure 2**.

Results

Through the database search, 492 initial papers were obtained. From them, 42 papers were eliminated as duplicates. After screening for applicability of the studies to humans and mention of the term "stem cells" in the title or abstract, 37 articles were selected for systematic analysis. To this number, 2 articles from external research were

Table 1. Analysis of articles

Author (year)	Country	Regenerative therapy	Types of regenerated hair cells (HCs)	Species (translational)	Anatomical route of cochlear transplantation
Li et al. [7] 2004	USA	cellular therapy	cochlear	birds mice	round window
López-Schier [50] 2004	USA	cellular therapy	cochlear	birds mice (humans)	
Parker & Cotanche [56] 2004	Switzerland	cellular therapy	cochlear	birds mice	
Hu et al. [49] 2005	Switzerland	cellular therapy gene therapy	cochlear	mice	tympanic scala (cochleostomy)
Hu & Ulfendahl [6] 2006	USA	cellular therapy	cochlear	birds mice	tympanic scala vestibular scala
Martinez-Monedero & Edge [27] 2007	USA	cellular therapy gene therapy	cochlear vestibular	mice	tympanic scala modiolus
Oshima et al. [11] 2007	USA	cellular therapy gene therapy	cochlear vestibular	rabbits mice (humans)	
Edge & Chen [23] 2008	USA	cellular therapy gene therapy	cochlear vestibular	birds mice fish	

Table 1 continued. Analysis of articles

Stem cells (SCs) classification	Study limitations	Findings and suggestions
embryonic neural bone marrow inner ear	Need for more studies in the area (cochlear anatomy). Correct incision procedure at the specific cochlear site. Immunological barriers; possibility of immunorejection (histological incompatibility). Potential use of immunosuppressants to overcome histocompatibility. Tumor formation.	SCs – may have applicability in neurodegenerative diseases such as Parkinson's and others such as diabetes. Injection of neural SCs. Possibility of the combined use of anti-rejection drugs with cell therapy so that this situation does not occur. Combination of cell therapy, gene therapy and pharmacological therapy.
neural inner ear	Tumor formation (uncontrolled cell cycle/ excessive proliferation). Need to control the orientation of new cHCs so as not to incur bad results.	Neural SCs have already generated new cHCs in the affected ear in mice.
neural inner ear embryonic hematopoietic (bone marrow)	Ethical considerations (use and destruction of human embryos). Tumor formation (after transplantation).	Isolating SCs from one's own ear may be a benefit for the treatment of degeneration. Neural SCs – therapeutic potential for hearing loss. May be preferred for treatment of neuropathy and disorders of the VIII cranial nerve.
embryonic neural	Need for more studies and results in the area; understand cell survival and implantation.	Neural SCs have possibly better outcomes than embryonic SCs, due to the ease of differentiation into cells of functional interest to the auditory system. Neural SCs can migrate to important functional structures such as the mature inner ear (along the cochlea). Combination of cell therapy and gene therapy would have greater applicability for auditory cell regeneration.
embryonic neural	Embryonic SCs: tumor formation. Administration of antibiotics (reduce/control risk of rejection and inflammation). Possibility of benign (teratomas) and malignant (teratocarcinoma) tumor formation; uncontrolled proliferation. More research needed in the area; more tissues, applicability, sources, cell differentiation.	CIs – need for functional afferent neurons for greater success. Embryonic SCs – can generate all types of cells. Neural SCs – ability to restructure afferent neurons. Potential ability to differentiate into neurons and glia cells during normal development and after transplantation into the nervous system. Seems to be a good bet in regenerative therapy.
embryonic	Type of cell choosen. Timing of infusion after damage. Differentiation state.	Neural replacement is very important in the success of cHC regeneration.
inner ear	Postnatal decrease of SCs at the ear level is apparently the main reason for the inability of mammals to regenerate their hearing organ after damage.	For the future, this may involve the mechanisms of action being differentiated at the cochlear and vestibular level of their SCs – discriminating between simple loss of SCs (or their ability to proliferate) and their potential as an active mechanism of repression, as well as how cochlear and vestibular cHCs act.
embryonic exogenous mesenchymal (bone marrow)	Choice and differentiation status of SCs when transplanted. It has been tricky to regenerate cHC with cells transplanted from sources other than the ear.	Embryonic SCs – successful to differentiate into neurons and into cHCs. Mesenchymal SCs (bone marrow) – have been used as growth factors and cellular markers for the regeneration of cHCs. SC transplantation has higher chances compared to endogenous regeneration. Challenge: good ordering and innervation of the cHC.

Table 1 continued. Analysis of articles

Author (year)	Country	Regenerative therapy	Types of regenerated hair cells (HCs)	Species (translational)	Anatomical route of cochlear transplantation
Pauley et al. [46] 2008	USA	cellular therapy gene therapy	cochlear vestibular	birds mice (humans)	
Vlastarakos et al. [48] 2008	Greece	cellular therapy gene therapy	cochlear	birds mice (humans)	rosenthal canal scala media tympanic scala round window modiolus perilymphatic perforation
Brigande & Heller [47] 2009	USA	cellular therapy gene therapy pharmacological therapy	cochlear vestibular	birds mice	scala media
Jongkamonwiwat et al. [35] 2010	United Kingdom	cellular therapy gene therapy	cochlear vestibular	birds mice	scc lateral scala media tympanic scala modiolus
Felipe et al. [54] 2011	Spain	cellular therapy gene therapy	cochlear	birds mice (humans)	rosenthal canal tympanic scala modiolus auditory nerve

Table 1 continued. Analysis of articles

Stem cells (SCs) classification	Study limitations	Findings and suggestions
embryonic adult: – inner ear, – hematopoietic, – neural, – olfactory	Possibility of immunorejection (histological incompatibility). Ethical considerations (use of embryos). Tumor formation. Better understanding of the auditory epithelium. Complex architecture of the cochlea and orientation of the new cHCs.	Embryonic SCs – studied for neurodegenerative diseases. Induced pluripotent SCs – have many characteristics of embryonic and adult SCs. Induced pluripotent SCs appear to be one of the most promising cell sources for auditory regenerative therapy.
embryonic bone marrow neural	Surgical procedure – possibility of intrascalar bleeding (small amount) after drilling the cochlear base. Middle ear infection. Prophylactic administration of antibiotics is a standard part of the surgical procedure (reducing risk of inflammation). Possibility of immunorejection (histological incompatibility). More studies are needed in the area, in terms of mechanisms, human genome, strategies.	Immunosuppression prior to the surgical procedure, to reduce the risk of rejection. Neural SC transplantation – can adopt the phenotypes, morphology, inner hair cells, and outer hair cells. Embryonic neuron-derived SCs – potential for synapse formation with cHCs and reinnervation of auditory epithelium. Scala media – survival of implanted cHCs. Modiolus – strategy used for regeneration of the spiral ganglion.
embryonic neural inner ear	The path taken by SCs to reach the cochlea. Anatomical limitations of the cochlea and organ of Corti (access to structures) and cochlear chemical composition (>K+ content). Activation of SCs and that they are correct in number at the correct site of damage. Possibility of immunorejection (histological incompatibility). Tumor formation. Need for further studies in the area (anatomical and histological).	The goal is to try to counteract tumor formation and the risk of rejection.
embryonic mesenchymal (bone marrow) neural inner ear induced pluripotent	Condition of the host tissue. Choice of transplantation route. Embryonic SCs – immunological barriers – possibility of immunorejection (histological incompatibility). Ethical considerations. Need for further studies in the area of human SCs.	Source of SCs from inner ear is the utricle (has some regenerative capacity) and cochlea (more complex procedure) – up to 3 weeks after birth. CIs would be complementary to therapies. Best results with electrical stimulation through CIs (spiral ganglion). Embryonic SCs – control of potassium homeostasis and cochlear generative potential. Induced pluripotent SCs – is more indicated for immunosuppression. Perilymphatic transplantation is less traumatic and can be done by cochleostomy or through the round window. Combination of SCs and CIs still needs more studies. Transplantation via modiolus to gain direct access to the Rosenthal canal – best method to access the spiral ganglion.
induced pluripotent exogenous	Anatomical limitations (access to structures) and cochlear chemical composition (>K+ content). Transplanting SCs into such a complex structure with the organ of Corti. SCs transplanted through the tympanic scala may have low survival ratio. If it is necessary to inject SCs multiple times, infection may result. Tumor formation. Need for more studies in the area.	ABR to prove electrophysiological thresholds. Induced pluripotent SCs with promising results for auditory regeneration.

Table 1 continued. Analysis of articles

Author (year)	Country	Regenerative therapy	Types of regenerated hair cells (HCs)	Species (translational)	Anatomical route of cochlear transplantation
Parker [25] 2011	USA	cellular therapy gene therapy	cochlear vestibular	birds mice fish (humans)	
Okano & Kelley [22] 2012	USA	cellular therapy gene therapy pharmacological therapy	cochlear	birds mice fish (humans)	rosenthal canal scala media tympanic scala
Hu & Ulfendahl [28] 2013	USA	cellular therapy gene therapy pharmacological therapy	cochlear vestibular	mice (humans)	tympanic scala vestibular scala
Almeida-Branco et al. [8] 2014	Spain	cellular therapy gene therapy pharmacological therapy	cochlear	birds mice	rosenthal canal tympanic scala modiolus (introduction into perilymph and endolymph)
Bas et al. [42] 2014	USA	cellular therapy gene therapy pharmacological therapy	cochlear vestibular	mice (humans)	
Park et al. [17] 2014	USA	cellular therapy	cochlear	mice	scala media tympanic scala
Lyon [43] 2017	USA	cellular therapy pharmacological therapy	cochlear	mice (humans)	middle ear

Table 1 continued. Analysis of articles

Stem cells (SCs) classification	Study limitations	Findings and suggestions
embryonic adult	Limited potential due to widespread differentiation into cells of the organ of Corti. embryonic SCs and their resources are not yet evidence with regard to auditory regeneration; however the results seem encouraging. Tumor formation.	Mention retinoic acid. Neural SCs – regenerate nervous tissue such as neurons and motor function. Cochlear markers faster than embryonic and mesenchymal SCs. They are faster because they are closer to cochlear tissue. Neural and mesenchymal SCs – maintain the ability to migrate throughout the injured cochlea. Neural and embryonic SCs – retain the ability to differentiate into neurons. So this alternative would be suitable for neurodegenerative diseases such as Alzheimer's and Parkinson's.
embryonic adult: – tissue specific – hematopoietic – mesenchymal (bone marrow) induced pluripotent	Anatomical limitations of the cochlea (access to structures) and cochlear chemical composition (>K+ content). Basilar membrane may compromise the approach by scala. SC to ear transplantation by injection seems insufficient to regenerate a substantial number of cHCs and thus will not have the capacity to form a functional auditory epithelium.	Induced pluripotent SCs – overcome the possibility of immunorejection and ethical considerations. Transplants of endogenous SCs will lead to further regeneration of the OHC. Alternative strategies to use SCs with a spiral ganglion regeneration or with conventional therapies such as CI must be equated for the best benefit of the patient.
embryonic neuron-derived mesenchymal neural inner ear induced pluripotent	Ethical considerations. Access to the vestibule and cochlea. Anatomical limitations and cochlear chemical composition. Existence of neurodegeneration. Molecular mechanisms still undetermined.	It is related to the CI surgery. Transplantation technique via tympanic scala has less trauma to the cochlea. Mesenchymal SCs – in vitro regeneration and proliferation. Neural SCs – can restore hearing via exogenous transplantation.
embryonic adult: – hematopoietic – mesenchymal (bone marrow) neural inner ear induced pluripotent	Anatomical limitations of the cochlea and organ of Corti (access to structures) and cochlear chemical composition (> K+ content). Possible loss of endolymph from the cochlear canal due to surgery. Further studies in the area are needed.	Combination of cell therapy, gene therapy, and CIs seem interesting for the treatment of sensorineural hypoacusis. CIs electrical stimulation would be complementary to therapies (cell + gene + pharm = better mid-term results). Spiral ganglion regeneration and replacement would be one of the main points to restore hearing function. Induced pluripotent SCs – limit cell differentiation. Safest for cHC and cochlear neurons.
embryonic mesenchymal (bone marrow and olfactory) induced pluripotent	Embryonic and induced pluripotent SCs – ethical and safety considerations. Choosing the most appropriate olfactory SCs, due to the existence of a wide variety of them in the epithelium. Need for further studies.	Mesenchymal SCs (olfactory) – studies describe the efficiency of the cells' potential for successful brain regeneration. But there is a need for further studies for better conclusions.
exogenous SCs	Anatomical limitations (access to structures) and cochlear chemical composition (>K+ content in scala media).	The injection of cHCs into the tympanic scala means that they can survive, but they are unable to pass the basilar membrane into the auditory epithelium.
does not specify (speaks generally)	Reduced number of differentiation in cHC. Need for regeneration of both inner and outer cHCs. Regenerating cochlear potentials in the appropriate locations.	Cellular markers of the cochlea are similar to those of intestinal SCs.

Table 1 continued. Analysis of articles

Author (year)	Country	Regenerative therapy	Types of regenerated hair cells (HCs)	Species (translational)	Anatomical route of cochlear transplantation
Mittal et al. [51] 2017	USA	cellular therapy gene therapy pharmacological therapy	cochlear	fish mice (humans)	scala media tympanic scala modiolus
Mahmoudian-Sani et al. [32] 2017	Iran	cellular therapy gene therapy	cochlear	mice	perilymphatic perforation
Simoni et al. [39] 2017	Italy	cellular therapy	cochlear	mice (humans)	tympanic scala
Diensthuber & Stöver [2] 2018	Germany	cellular therapy gene therapy pharmacological therapy	cochlear	birds mice (humans)	
Chen et al. [33] 2018	China	cellular therapy gene therapy	cochlear	mice	round window
Lee & Park [41] 2018	South Korea	cellular therapy gene therapy	cochlear	mice	middle scala tympanic scala
Takeda et al. [34] 2018	USA	cellular therapy gene therapy	cochlear	mice (humans)	round window modiolus
Tang et al. [40] 2018	China	cellular therapy	cochlear	mice	
Hyakumura et al. [31] 2019	Australia	cellular therapy	cochlear	mice (humans)	modiolus

Table 1 continued. Analysis of articles

Stem cells (SCs) classification	Study limitations	Findings and suggestions
embryonic umbilical cord mesenchymal (bone marrow) neural olfactory	It is not really clear that SCs directly produce cHC. Tumor formation and apoptosis. More studies are needed in the area – new strategies will emerge.	Cellular regeneration of the ear for Usher syndrome. The SC option may be the future of ex vivo expansion of patient's own SCs (autologous) and their reintroduction into the injured tissue. Developing cHCs from cochlea support cells – most promising method to regenerate cHCs.
mesenchymal – bone marrow – adipose tissue – olfactory tissue – umbilical cord		Bone marrow SCs have the best results among mesenchymal SCs.
embryonic hematopoietic mesenchymal (umbilical cord) neural inner ear induced pluripotent	Affecting the complex cytoarchitecture of the cochlea and residual hearing function. Recover tonotopic cochlear capacity.	Inner ear SCs grow up with good prospects of restoring hearing.
embryonic adult: – mesenchymal (bone marrow) – inner ear induced pluripotent	Ethical considerations (use of human embryos). Further human studies needed.	Induced pluripotent SCs – hold great promise for hearing regeneration. CI stimulation would be complementary to therapies (such as growth factors).
embryonic induced pluripotent		Induced pluripotent SCs – obtained from human urine. Embryonic SCs induced from urine to pluripotent induced SCs.
embryonic induced pluripotent	Anatomical limitations and cochlear chemical composition. Tumor formation – differentiation and uncontrolled development. Expensive procedures.	CIs would be complementary to therapies.
embryonic mesenchymal (bone marrow) induced pluripotent	More studies in humans needed. Anatomical limitations (access to structures) and cochlear chemical composition (>K+ content in the middle range). Time factor – long-term effects of treatment are not known, both at the level of CCC (survival and behavior). Tumor formation. Differentiation status of SCs when transplanted. Access route to the cochlea and its physical barriers (Reissner's and basilar membrane).	Pluripotent SCs (embryonic and induced pluripotent SCs) seem to be the most suitable to proceed to ear regeneration therapy. Better therapeutic results in the tympanic scala approach compared to lateral or posterior semicircular canal. Good completion of transplantation for the inner ear.
mesenchymal (bone marrow) neural	Create a suitable microenvironment to carry out the research and results.	Electrical stimulation (by electric field) to regulate cell behavior. Electrical stimulation through CIs that promotes neural SCs to differentiate into neurons.
embryonic neural pluripotent (human)		Studies with pluripotent SCs. Co-cultures concept – meaning manipulating an environment to resemble *in vivo* characteristics (microenvironment).

Table 1 continued. Analysis of articles

Author (year)	Country	Regenerative therapy	Types of regenerated hair cells (HCs)	Species (translational)	Anatomical route of cochlear transplantation
Roccio & Edge [38] 2019	Switzerland	cellular therapy gene therapy pharmacological therapy	cochlear vestibular	mice (humans)	
Xia et al. [30] 2019	China	cellular therapy gene therapy pharmacological therapy	cochlear vestibular	mice (humans)	cochlear lateral wall
Waqas et al. [21] 2020	Pakistan	cellular therapy pharmacological therapy	cochlear	mice	scala media tympanic scala (via round window)
Zhang et al. [37] 2020	China	cellular therapy gene therapy	cochlear	birds fish mice	
Zine et al. [55] 2021	France	cellular therapy gene therapy pharmacological therapy	cochlear	fish mice (humans)	cochlear nerve scala media tympanic scala modiolus tympanic scala (intraperilymphatic and intraendolymphatic)
Maharajan et al. [57] 2021	South Korea	cellular therapy gene therapy pharmacological therapy	cochlear	mice (humans)	
Guo et al. [58] 2021	China	cellular therapy gene therapy pharmacological therapy	cochlear	mice	
Kempfle [53] 2021	USA	cellular therapy gene therapy pharmacological therapy	cochlear	mice (humans)	round window (transtympanic) cochleostomy

Table 1 continued. Analysis of articles

Stem cells (SCs) classification	Study limitations	Findings and suggestions
induced pluripotent	Complex architecture of the sensory epithelium. Surgical access.	
embryonic neural induced pluripotent	Long-term effects of treatment are not known, both at the level of cHC (survival and behavior). Anatomical limitations (access to structures). More studies needed in the area.	CIs would be complementary to electrical stimulation therapies. Embryonic, neural, and induced pluripotent SCs – relate to cHCs and spiral ganglion.
exogenous endogenous embryonic induced pluripotent	Barriers with tight junctions. Ethical considerations. Tumor formation. Correct incidence on specific cochlear tissue and insufficient number of SCs residing in the inner ear. Expensive treatment.	Induced pluripotent SCs – obtained from human urine.
inner ear	More studies are needed in the area on growth factors and signals.	cHCs can be regenerated by SCs, genes, and signaling regulation. Need to inhibit apoptosis, analyze other genes and maturation – should be done in the future.
embryonic induced pluripotent pluripotent	Surgical delivery routes play a huge technical factor in the cochlea. Deliver the cells into the anatomic target. Establish precise cell injection through the cochlea, while minimizing surgical trauma and hearing loss. Current limitations to the use of human induced pluripotent SCs – extended in vitro period of culture, reproducibility, variable efficiency of tissue derivation, incapacity to generate cochlear tissues. Need for more studies in the area and humans.	Advanced in research and recent studies in human induced pluripotent SCs. Approach by intraperilymphatic injection, intraendolymphatic injection, modiolar, and cochlear nerve injection. In the close future, a possible regeneration of inner ear can include network vascularization and integration into microfluidic chips.
embryonic induced pluripotent mesenchymal	Successful delivery of mesenchymal SCs to the target sites, and necessary of a suitable microenvironment for survival and migration. Transplanted mesenchymal SCs – may cause immunorejection, inflammation and tumor formation.	Different mesenchymal SCs isolation methods with almost similar functional characteristics.
autophagy (does not include SC)	Complexity of autophagy mechanisms. Current autophagy research – limited to cell lines, explants and animals, and few clinical trials have been examined. Although excessive autophagy can lead to cell death under some conditions. Need for more studies in the area.	Some proteins and mRNAs participate in the autophagy and can make them potential targets for treatment of sensorioneural hearing loss.
embryonic induced pluripotent mesenchymal	Need for more studies in the area and humans.	Endoscopic ear surgery provides a minimally invasive approach to the inner ear for regenerative therapies. Possible routes: transtympanic delivery (indirect drug application into the round window membrane – in patients with residual hearing), and through the round window membrane or via cochleostomy (performed with the endoscope to target in patients without residual hearing).

Table 1 continued. Analysis of articles

Author (year)	Country	Regenerative therapy	Types of regenerated hair cells (HCs)	Species (translational)	Anatomical route of cochlear transplantation
Kwan & White [59] 2021	USA	cellular therapy gene therapy	cochlear	mice (humans)	
Lee & Waldhaus [4] 2022	USA	cellular therapy gene therapy pharmacological therapy	cochlear vestibular	birds fish mice (humans)	

added, resulting in a final number of 39 articles. The flow chart following the PRISMA guidelines [37] is shown in **Figure 2**.

The distribution of papers in the databases shows a gradual increase in number over the years, with the greatest number being obtained in the period 2005–2010 and in the last half of the decade (2015–2022). The geographical distribution (by number of papers) revealed a large contribution from the United States of America (19), followed by China (5), Switzerland (3), Spain (2), South Korea (2), Australia (1), Iran (1), Pakistan (1), France (1), Greece (1), Italy (1), Germany (1), and UK (1).

Discussion

The main focus in cochlear hair cell regeneration research is cHC regeneration [21,37], sometimes also considering vestibular (vHC) regeneration [30,38,39]. The challenge for the research covered in this review was to decide upon the most effective method for such regeneration within the human ear, since it has complex microstructure and physiology.

In regenerative therapies, there are some studies of cell therapy alone [40,41]. Others combine cell therapy with gene therapy [3,42] or with pharmacological therapy [43,44]. Finally, some studies consider all three – cell therapy, gene therapy, and pharmacological therapy [8,39].

The goal of SC-based replacement therapy in sensorineural hearing loss is to replace the lost cHCs or neurons of the spiral ganglion, with the biggest challenge being precise targeting without disrupting the cochlear architecture and damaging residual hearing function [45,46].

The complex architecture of the sensory epithelium and its difficult surgical access are anatomical limitations [39]. Transplantation involves overcoming physical barriers within the cochlea: Reissner's membrane, the basilar membrane, the organ of Corti, and the vestibule [29–31,35]. The importance of a correct incision at the specific cochlear site is vital to safe SC therapy [30]. Moreover, the growth, differentiation, and orientation of the new SCs, plus the recovery of the incisional regeneration site, are further considerations [44,46].

This review revealed that the use of embryonic SCs can trigger uncontrolled cell formation and development, with increased risk of tumor formation [30,42]. There is also the possibility of histological incompatibility (i.e. immunorejection) [3,47]. To overcome these immunological barriers, the use of anti-rejection drugs (immunosuppressants) combined with cell therapy is the favoured solution [7,48,49], and the use of autologous grafts may also help to circumvent this, as well as prophylactic antibiotic administration to reduce infection risk [49].

All procedures in regenerative therapies, specifically cell therapy, are costly, and it is also difficult to create a suitable microenvironment [3,41,42]. Therapies should be applied according to the cochlear and neural reserve of the patient [8], keeping in mind that neurodegeneration is a barrier to the entire regenerative process [6].

Lastly, the narrative review revealed that CIs can complement SC therapies, promoting electrical stimulation, and this approach may show better results in the future [30,42]. Electrical stimulation is one of the most important factors in regulating cell behavior; it influences cell proliferation, differentiation, and migration. Thus, in the future CIs have a potential role to assist SCs [30,41].

The long-term effects of treatment on both behavior and survival of cHCs are not yet known [31,35]. More studies are needed, namely of various progenitor cell populations, implications, specific factors, as well as combination and complementary therapies [3,23].

Sources of stem cells

This review looked systematically at the sources of SCs (**Table 1**). The following are the most relevant to auditory regeneration.

Table 1 continued. Analysis of articles

Stem cells (SCs) classification	Study limitations	Findings and suggestions
induced pluripotent pluripotent	Spontaneous lineage conversion – not observed after damage (in mature mammalian cochlea). Need for more studies in the area and humans.	Advanced in research and recent studies. Neonatal and even mature SCs can be genetically manipulated.
induced pluripotent mesenchymal neural pluripotent tissue specific	Limited regenerative capacity and the potential to isolate SC during mice postnatal development. Limitations of the pluripotent SCs-based approaches – cellular output of the present-day protocols. Anatomical limitations of the cochlea and organ of Corti (access to structures) and cochlear chemical composition (>K+ content). Need for more studies in the area and humans (various in vivo and *in vitro* approaches in study).	Functional hair cell regeneration: non-mammalian vertebrate (e.g. birds and fish). Current research has focused on tissue specific SCs and pluripotent SCs. Less explored research: neural SCs and mesenchymal SCs. The continued study and use of human induced pluripotent SCs can open the way to understanding more complex diseases, like Waardenburg syndrome.

Neural SCs and embryonic SCs

Retain the ability to differentiate into neurons, and this approach may be suitable for neurodegenerative diseases such as Alzheimer's and Parkinson's. Neural SCs regenerate nervous tissue such as neurons and motor function, expressing cochlear markers faster than embryonal and mesenchymal SCs. Neural SCs are the fastest to regenerate because of their proximity to cochlear tissue [26]. Moreover, neural SCs were among the first to generate new cHCs in the ear [51]. Their use, together with electrical stimulation via CIs, leads to their differentiation into neurons, showing evidence that they can restore hearing via exogenous transplantation [29,35]. These SCs have the ability to migrate to important functional structures such as the mature inner ear [50].

Inner ear SCs

May in future be capable of restoring hearing and beneficial for treating degeneration found in hearing loss, neuropathy, or disorders of the VIII cranial region [25].

Embryonic and induced pluripotent SCs

Embryonic and induced pluripotent SCs related to cHCs and the spiral ganglion are thought to be the most likely to lead to therapeutic success [31]. Most studies state that pluripotent SCs (embryonic and induced pluripotent SCs) seem to be the most suitable for ear regeneration therapy [38,52].

Possible routes for SC transplantation

Regarding the anatomical route of cochlear transplantation, perilymphatic transplantation is less traumatic and can be done by cochleostomy or through the round window [37]. Endolymph loss through the cochlear canal is possible, and bleeding is also possible due to surgery and cochlear perforation [8,49].

Developing cHCs from cochlear support cells seems one of the most promising methods for regeneration [53,54]. Alternative strategies propose using SCs for spiral ganglion regeneration together with conventional therapies such as a CI to maximise patient benefit [23]. Transplantation via the scala tympani is less traumatic to the cochlea and can be combined with CI surgery [29]. The modiolus approach can also be used at the same time as a CI operation, thus limiting the risk of residual hearing loss. Direct access to Rosenthal's canal might be the best method to access the spiral ganglion [37,55].

Overall, research into transplantation methods is still insufficient, with a need for further anatomical and histological studies [31,48,56], particularly in human tissues [10,35,54] and umbilical cord serum [57]. The question about full or partial recovery of tonotopic cochlear capacity remains to be answered [56,58].

A variety of signaling pathways, including Wnt, Notch, Hippo-YAP, Hedgehog, LIN28/Let7, key transcriptional factors (*Atoh1* or *Math1)*, and fibroblast growth factors, have been found to be involved in regulating hair cell development and regeneration by controlling the expression of various transcription factors [for review, 13].

Future research trends may involve differentiated action mechanisms at the cochlear and vestibular level, discriminating between simple loss of SCs (or their ability to proliferate) and their potential as an active regeneration mechanism [10]. The need for apoptosis inhibition, the analysis of other genes, and cell maturation must also be considered [38,55,59].

Despite ongoing challenges, embryonic and induced pluripotent SCs derived from inner ear cultures have already demonstrated potential for disease modelling and therapeutic trials. However, future continued research is required to achieve protocol optimisation and to improve applications and outcomes. The use of patient-derived cultures can facilitate the evaluation of gene therapy efficacy,

a possibility that has been trialled in other model systems, such as the eye [60].

The combined use of cell therapy and gene therapy (gene programming/editing technology) may have more promising results and strategic applicability, and are likely to become a future research trend. Regardless of the technology used, the majority of studies found support the use of pluripotent SCs. However, the continued threat of having pluripotent SCs becoming uncontrollable and inducing genetic damage and malignant cell growth is ever-present, and the potential and fate of these cells *in vivo* are under intense investigation.

Conclusions

Inner ear pathology and therapeutic developments have traditionally relied on animal models, which usually cannot completely recapitulate the human otic system. These challenges are now being partly addressed using induced pluripotent SCs in lab cultures, which generate the sensory epithelial-like inner ear tissues.

Developing pluripotent SCs (embryonic and induced pluripotent SCs) seems to be the most promising method for ear regeneration therapy for cHCs, inserted by cochleostomy or through the round window.

The combined use of cell therapy and gene therapy appears to be the most promising method. Moreover, advances in cHC regeneration with stem cells suggest there is a realistic potential to make the technique a useful future therapy.

For implementation, timing and safe procedures are crucial to achieving an effective protocol. Further investigation is required to determine whether transplanted cells can survive long term and contribute functionally. There is also the question of whether stimulation by a CI or hearing aid promotes survival and is needed in the (re)habilitation protocol.

The current technology market may act to hinder advances in regeneration of cHCs, with low investment partly because hearing loss can usually be safely and economically rehabilitated with devices like cochlear implants and hearing aids.

If new SC treatments are introduced, the role for an audiologist will remain, as people still require diagnosis and monitoring of their hearing, both pre- and post-treatment. The use of SCs might take some decades, but we should not fear innovation. Everything that can improve quality of life, specifically hearing health, must be part of our future solutions.

Author contributions

VC and DT were involved in the conceptualization and design of the study. VC conducted the article search and writing. FC and TP-B critically reviewed the study protocol and writing. All authors were involved in the revision of the manuscript and approved the final version.

Conflict of interest

The authors declare no competing interests.

References

1. Costa A, Sanchez-Guardado L, Juniat S, Gale J, Daudet N, Henrique D. Generation of sensory hair cells by genetic programming with a combination of transcription factors. J Dev, 2015; 142: 1948–59. https://doi.org/10.1242/dev.119149
2. Diensthuber M, Stöver T. Strategies for a regenerative therapy of hearing loss. HNO, 2018; 66(1): 39–46. https://doi.org/10.1007/s00106-017-0467-0
3. Durán-Alonso. Stem cell-based approaches: possible route to hearing restoration? WJSC, 2020; 12(6): 422–37. https://doi.org/10.4252/wjsc.v12.i6.422
4. Lee M, Waldhaus J. In vitro and in vivo models: what have we learnt about inner ear regeneration and treatment for hearing loss? Mol Cellular Neurosci, 2022; 120. https://doi.org/10.1016/j.mcn.2022.103736
5. Dufner-Almeida L, Cruz D, Netto R, Batissoco A, Oiticica J, Salazar-Silva R. Stem-cell therapy for hearing loss: are we there yet? BJORL, 2019; 85(4): 520–9. https://doi.org/10.1016/j.bjorl.2019.04.006
6. Hu Z, Ulfendahl M. Cell replacement therapy in the inner ear. Stem Cells Dev, 2006; 15(3): 449–59. https://doi.org/10.1089/scd.2006.15.449
7. Li H, Corrales C, Edge A, Heller S. Stem cells as therapy for hearing loss. Trends Mol Med, 2004; 10(7): 309–15. https://doi.org/10.1016/j.molmed.2004.05.008
8. Almeida-Branco M, Cabrera S, Lopez-Escamez J. Perspectivas para el tratamiento de la hipoacusia neurosensorial mediante regeneración celular del oído interno. Acta Otorrinolaringol Esp, 2014; 66(5): 286–95. https://doi.org/10.1016/j.otorri.2014.07.009
9. Hirschi K, Li S, Roy K. Induced pluripotent stem cells for regenerative medicine. Ann Rev Biomed Eng, 2014; 16: 277–94. https://doi.org/10.1146/annurev-bioeng-071813-105108
10. Monteiro L, Subtil J. Audiologia, som e audição das bases à clínica. 1st ed. Queluz, Portugal: Círculo Médico; 2018.
11. Oshima K, Grimm C, Corrales E, Senn P, Monedero R, Géléoc G, et al. Differential distribution of stem cells in the auditory and vestibular organs of the inner ear. JARO, 2007; 8(1): 18–31. https://doi.org/10.1007/s10162-006-0058-3
12. Gillespie L, Richardson R, Nayagam B, Wise A. Treating hearing disorders with cell and gene therapy. J Neural Eng, 2014; 11(6). https://doi.org/10.1088/1741-2560/11/6/065001
13. Qi J, Huang W, Lu Y et al. Stem cell-based hair cell regeneration and therapy in the inner ear. Neurosci Bull, 2024; 40: 113–26. https://doi.org/10.1007/s12264-023-01130-w
14. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 2006; 126(4): 663–76. https://doi.org/10.1016/j.cell.2006.07.024
15. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL. Induced pluripotent stem cell lines derived from human somatic cells. Science, 2007; 318(5858): 1917–20. https://doi.org/10.1126/science.1151526
16. Chen J-R, Tang Z-H, Zheng J, Shi H-S, Ding J, Qian X-D, et al. Effects of genetic correction on the differentiation of hair cell-like cells from iPSCs with MYO15A mutation. Cell Death Differ, 2016; 23(8): 1347–57. https://doi.org/10.1038/cdd.2016.16

17. Park Y-H, Wilson K, Ueda Y, Wong H, Beyer L, Swiderski D, et al. Conditioning the cochlea to facilitate survival and integration of exogenous cells into the auditory epithelium. Mol Ther, 2014; 22(4): 873–80. https://doi.org/10.1038/mt.2013.292
18. Needham K, Minter R, Shepherd R, Nayagam B. Challenges for stem cells to functionally repair the damaged auditory nerve. Expert Opin Biol Ther, 2013; 13(1): 85–101. https://doi.org/10.1517/14712598.2013.728583
19. Roccio M, Senn P, Heller S. Novel insights into inner ear development and regeneration for targeted hearing loss therapies. Hear Res, 2019; 397: 107859. https://doi.org/doi.org/10.1016/j.heares.2019.107859
20. Johnson K, Gagnon L, Tian C, Longo-Guess C, Low B, Wiles M, et al. Deletion of a long-range Dlx5 enhancer disrupts inner ear development in mice. J Genet, 2018; 208(3): 1165–79. https://doi.org/10.1534/genetics.117.300447
21. Waqas M, Us-Salam I, Bibi Z, Wang Y, Li H, Zhu Z, et al. Stem cell-based therapeutic approaches to restore sensorineural hearing loss in mammals. Neural Plast, 2020; 8829660. https://doi.org/doi.org/10.1155/2020/8829660
22. Okano T, Kelley M. Stem cell therapy for the inner ear: recent advances and future directions. Trends Amplif, 2012; 16(1): 4–18. https://doi.org/10.1177/1084713812440336
23. Edge A, Chen Z. Hair cell regeneration. Curr Opin Neurobiol, 2008; 18(4): 377–82. https://doi.org/10.1016/j.conb.2008.10.001
24. Müller U, Barr-Gillespie P. New treatment options for hearing loss. Nat Rev Drug Discov, 2015; 14(5): 346–65. https://doi.org/10.1038/nrd4533
25. Parker M. Biotechnology in the treatment of sensorineural hearing loss: foundations and future of hair cell regeneration. JSLHR, 2011; 54(6): 1709–31. https://doi.org/10.1044/1092-4388(2011/10-0149)
26. Rubel E, Furrer S, Stone J. A brief history of hair cell regeneration research and speculations on the future. Hear Res, 2013; 297: 42–51. https://doi.org/10.1016/j.heares.2012.12.014
27. Martinez-Monedero R, Edge A. Stem cells for the replacement of inner ear neurons and hair cells. Int J Dev Biol, 2007; 51(6–7): 655–61. https://doi.org/10.1387/ijdb.072372rm
28. Hu Z, Ulfendahl M. The potential of stem cells for the restoration of auditory function in humans. Regen Med, 2013; 8(3): 309–18. https://doi.org/10.2217/rme.13.32
29. Qiu Y, Qiu J. Stem cells: a new hope for hearing loss therapy. Adv Exp Med Biol, 2019; 1130: 165–80. https://doi.org/10.1007/978-981-13-6123-4_10
30. Xia M, Ma J, Sun S, Li W, Li H. The biological strategies for hearing re-establishment based on the stem/progenitor cells. Neurosci Lett, 2019; 711. https://doi.org/10.1016/j.neulet.2019.134406
31. Hyakumura T, McDougall S, Finch S, Needham K, Dottori M, Nayagam B. Organotypic cocultures of human pluripotent stem cell derived-neurons with mammalian inner ear hair cells and cochlear nucleus slices. Stem Cells Int, 2019; 8419493. https://doi.org/10.1155/2019/8419493
32. Mahmoudian-Sani M, Mehri-Ghahfarrokhi A, Hashemzadeh-Chaleshtori M, Saidijam M, Jami M. Comparison of three types of mesenchymal stem cells (bone marrow, adipose tissue, and umbilical cord-derived) as potential sources for inner ear regeneration. Int Tinnitus J, 2017; 21(2): 122–7. https://doi.org/10.5935/0946-5448.20170023
33. Chen J, Hong F, Zhang C, Li L, Wang C, Shi H, et al. Differentiation and transplantation of human induced pluripotent stem cell-derived otic epithelial progenitors in mouse cochlea. Stem Cell Res Ther, 2018; 9(1): 230. https://doi.org/10.1186/s13287-018-0967-1
34. Takeda H, Dondzillo A, Randall J, Gubbels S. Challenges in cell-based therapies for the treatment of hearing loss. TINS, 2018; 41(11): 823–37. https://doi.org/10.1016/j.tins.2018.06.008
35. Jongkamonwiwat N, Zine A, Rivolta M. Stem cell based therapy in the inner ear: appropriate donor cell types and routes for transplantation. Curr Drug Targets, 2010; 11(7): 888–97. https://doi.org/10.2174/138945010791320836
36. Zhang S, Qiang R, Dong Y, Zhang Y, Chen Y, Zhou H, et al. Hair cell regeneration from inner ear progenitors in the mammalian cochlea. Am J Stem Cells, 2020; 9(3): 25–35.
37. Moher D, Liberati A, Tetzlaff J, Altman D, Prisma Group. Preferred reporting items for systematic review and meta-analyses: the PRISMA Statement. Open Med J, 2009; 3(2): 123–30. https://doi.org/10.1016/j.jclinepi.2009.06.005
38. Roccio M, Edge A. Inner ear organoids: new tools to understand neurosensory cell development, degeneration and regeneration. J Dev, 2019; 146(17). https://doi.org/10.1242/dev.177188
39. Simoni E, Orsini G, Chicca M, Bettini S, Franceschini V, Martini A, et al. Regenerative medicine in hearing recovery. Cytotherapy, 2017; 19(8): 909–15. https://doi.org/10.1016/j.jcyt.2017.04.008
40. Tang M, Yan X, Tang Q, Guo R, Da P, Li D. Potential application of electrical stimulation in stem cell-based treatment against hearing loss. Neural Plast, 2018; https://doi.org/10.1155/2018/9506387
41. Lee M, Park Y. Potential of gene and cell therapy for inner ear hair cells. Biomed Res Int, 2018. https://doi.org/10.1155/2018/8137614
42. Bas E, Water T, Lumbreras V, Rajguru S, Goss G, Hare J, et al. Adult human nasal mesenchymal-like stem cells restore cochlear spiral ganglion neurons after experimental lesion. Stem Cells Dev, 2014; 23(5): 502–14. https://doi.org/10.1089/scd.2013.0274
43. Lyon J. Hearing restoration: a step closer? JAMA, 2017; 318(4): 319–20. https://doi.org/10.1001/jama.2017.5820
44. Gunewardene N, Dottori M, Nayagam B. The convergence of cochlear implantation with induced pluripotent stem cell therapy. Stem Cell Rev, 2010; 8: 741–54.
45. Revoltella R, Papini S, Rosellini A, Michelini M, Franceschini V, Ciorba A, et al. Cochlear repair by transplantation of human cord blood CD133+ cells to nod-scid mice made deaf with kanamycin and noise. Cell Transplant, 2008; 17: 665–78. https://doi.org/10.3727/096368908786092685
46. Pauley S, Kopecky B, Beisel K, Soukup G, Fritzsch B. Stem cells and molecular strategies to restore hearing. Panminerva Med, 2008; 50(1): 41–53.
47. Brigande J, Heller S. Quo vadis, hair cell regeneration? Nat Neurosci, 2009; 12(6): 679–85. https://doi.org/10.1038/nn.2311
48. Vlastarakos P, Nikolopoulos T, Tavoulari E, Kiprouli C, Ferekidis E. Novel approaches to treating sensorineural hearing loss. Auditory genetics and necessary factors for stem cell transplant. Med Sci Monit Basic Res, 2008; 14(8): 114–25.
49. Hu Z, Wei D, Johansson C, Holmström N, Duan M, Frisén J, Ulfendahl M. Survival and neural differentiation of adult neural stem cells transplanted into the mature inner ear. Exp Cell Res, 2005; 302(1): 40–7. https://doi.org/10.1016/j.yexcr.2004.08.023
50. López-Schier H. Regeneration: did you hear the news? Curr Biol, 2004; 14(3): 127–8.
51. Mittal R, Nguyen D, Patel A, Debs L, Mittal J, Yan D, et al. Recent advancements in the regeneration of auditory hair cells and hearing restoration. Front Mol Neurosci, 2017; 10: 236. https://doi.org/10.3389/fnmol.2017.00236

52. Connolly K, Anai Gonzalez-Cordero A. Modelling inner ear development and disease using pluripotent stem cells: a pathway to new therapeutic strategies. Dis Model Mech, 2022; 15(11): dmm049593. https://doi.org/10.1242/dmm.049593
53. Kempfle J. Endoscopic-assisted drug delivery for inner ear regeneration. Otolaryngol Clin North Am, 2021; 54(1). https://doi.org/10.1016/j.otc.2020.09.022
54. Felipe M, Redondo A, García-Sancho J, Schimmang T, Durán-Alonso M. Cell- and gene-therapy approaches to inner ear repair. Histol Histopathol, 2011; 26(7): 923–40. https://doi.org/10.14670/HH-26.923
55. Zine A, Messat Y, Fritzsch B. A human induced pluripotent stem cell-based modular platform to challenge sensorineural hearing loss. Stem Cells, 2021; 39: 697–706. https://doi.org/10.1002/stem.3346
56. Parker M, Cotanche D. The potential use of stem cells for cochlear repair. Audiol Neurotol, 2004; 9(2): 72–80. https://doi.org/10.1159/000075998
57. Maharajan N, Cho GW, Choi JH, Jang CH. Regenerative therapy using umbilical cord serum. In Vivo, 2021; 35(2): 699–705. https://doi.org/10.21873/invivo.12310
58. Guo L, Cao W, Niu Y, He S, Chai R, Yang J. Autophagy regulates the survival of hair cells and spiral ganglion neurons in cases of noise, ototoxic drug, and age-induced sensorineural hearing loss. Front Cell Neurosci, 2021; 15: 760422. https://doi.org/10.3389/fncel.2021.760422
59. Kwan K, White P. Understanding the differentiation and epigenetics of cochlear sensory progenitors in pursuit of regeneration. Curr Opin Otolaryngol Head Neck Surg, 2021; 29(5): 366–72. https://doi.org/10.1097/moo.0000000000000741
60. Kruczek K, Qu Z, Gentry J, Fadl BR, Gieser L, Hiriyanna S, et al. Gene therapy of dominant CRX-leber congenital amaurosis using patient stem cell-derived retinal organoids. Stem Cell Rep, 2021; 16: 252–63. https://doi.org/10.1016/j.stemcr.2020.12.018