J. of Applied Tissue Engineering
2018; 5(4): 1-9

Review Article

A Review on Inner Ear Tissue Engineering

Ameneh Mehri[1], Ali Shojaeian[1], Leila Rezakhani[*2]

1-Department of Molecular Medicine, School of Advanced Technologies, Shahrekord University of Medical Sciences, Shahrekord, Iran
2-Department of Tissue engineering, School of Advanced Technologies, Shahrekord University of Medical Sciences, Shahrekord, Iran Shahrekord, Iran

Received: 10 July 2018/ Accepted: 15 December 2018

Abstract:

Ear defects and hearing loss are still one of the challenging problems in medicine. Tissue engineering approaches are considered as promising and efficient tools for such problems. Tissue engineering is considered a newly appearing biomedical technology, which could repair and regenerate inadequate or injured tissues. It utilizes the principles from the areas of cell biology and transplantation, materials science and engineering to treat or replace injured tissues. Different types of cell sources, growth factors or signals, materials or scaffolds and different methods have been studied to repair or replace different parts of ear and restore its function and hearing. In this review we present recent such elements studied or proposed for inner ear reconstruction.

Key words: Tissue engineering, Inner ear, Hearing loss

Introduction

The ear in addition to the role played in beauty and face form, is the organ of hearing and, in mammals, balance. In mammals, the ear is usually described as having three parts; the outer ear, middle ear (tympanic cavity) and the inner ear. The outer ear consists of the pinna or auricle, the ear canal (External auditory canal or tube that connects the outer ear to the inside or middle ear) and tympanic membrane (also called the eardrum). The tympanic membrane divides the external ear from the middle ear. Middle ear or tympanic cavity consists of ossicles; three small bones that are connected and transmit the sound waves to the inner ear and called: Malleus, Incus and Stapes and Eustachian tube. Eustachian tube links the middle ear with the back of the nose. The eustachian tube helps to equalize the pressure in the middle ear. Equalized pressure is needed for the proper transfer of sound waves. Inner ear, consists of Cochlea (contains the nerves for hearing), Vestibule (contains receptors for balance) and Semicircular canals (contain receptors for balance) (1). All parts of ear have own functions and performance that finally cause to hearing and balance. Any damage to any part of ear causes different impairment of its functions. These injuries can be genetically or congenital or acquired by trauma, accident or by disease or tumor. Due to the complex shape and structure of the ear and its important function in hearing and balance,

*Corresponding Authors: Leila Rezakhani
Email: Leila_rezakhani@yahoo.com

reconstruction and restoration of it, is very important and challenging. Recently, tissue engineering approaches have been considered for the reconstruction of the ear (2). Tissue engineering can be effective in restoring and repairing various parts of the ear. In this review we introduce recent tissue engineering approaches in inner ear reconstruction.

Cell sources in inner ear tissue engineering

Some of cell sources applied in the research of inner ear regeneration are as follows: Endogenous inner ear Stem Cells, Embryonic Stem Cells (ESCs) and iPS, Adult Stem Cells, Limbal Epithelial Stem cells(LESC), Hair follicle stem cells, Olfactory epithelium, Amniotic fluid-derived stem cells(AFS), Mesenchymal Stem Cells (MSCs) and Neural Stem Cells.

Endogenous Inner Ear Stem Cells

However adult stem cells appear to be present in the inner ear (4), they may be difficult, if not impractical , to get without the damage of the very organ that they ought to restore and they are not likely to be beneficial in human therapies (5). In a culture system, stem cells were isolated from the utricle of the vestibular organ of a mouse and were differentiated into neurons in which nestin and tubIII were expressed by differentiation of the spheres (6).

Embryonic Stem Cells (ESCs) and iPS

Nowadays, for the substitution of auditory neurons, embryonic stem cells were conveyed into the inner ear (7-17). In previous studies, ESCs were assayed to restitute auditory function by generating neural progenitors and sensory auditory neurons with the possibility of connections to hair cells (18-23). In 2006, mouse embryonic stem cells (mESCs) were placed into guinea pig cochlea and a poor survival was detected. Based on neurofilament immunolabeling, a protein of the surviving mESCs demonstrated a neuronal phenotype (9). Hair cell-like phenotype was applied by Rivolta et al.,when cultured “in vitro” , in order to induce mESCs to a variety of inner ear-like phenotypes (24). In 2004 mESCs and a neuronal co-graft of dorsal root ganglion tissue were co-transplanted into guinea pig cochlea to study the impact of in vivo stem cell differentiation. The results showed that following co-graft placement , the survival of TUJ1 immunolabeled stem cells was improved (10).

Adult Stem Cells

Some of the advantages of the adult stem/progenitor cells made them as an appropriate option for autologous transplantation. Furthermore, these stem cells have fewer tumourigenic properties compared to embryonic stem cells. The immune system, hematopoietic system, skin and hair follicles, finger nails, the eye and even olfactory system are a few examples of organ systems with adult stem cells (5).

Limbal Epithelial Stem cells (LESC)

Cultured LESCs are one of the adult stem cell populations which may have been effectively used for medical treatment (5).

Hair Follicle Stem Cells

One way to obtain multipotent neural crest-derived stem cells is the hair follicle. It has been shown that whisker follicle stem cells of mice could differentiated into neurons, smooth muscle cells, schwann cells and melanocytes (5).

Olfactory Epithelium

According to the high potential of olfactory epithelium in regeneration of olfactory receptor neurons, it appears these cells can be used to increase the survival of neurons in auditory cells by trophic factors secretion or by the expression of a supportive protein matrix (2, 22, 23).

Amniotic Fluid-derived Stem Cells (AFS)

Several in vitro studies have demonstrated that, under specific conditions, amniotic fluid-derived stem cells are capable of differentiating into neuron-like cells (25-27).The directional differentiation of the inner ear stem cells in the derived feeder layer of AFS cell have been investigated in the absence of cytokines in order to clarify the fundamental mechanism (28).

Mesenchymal Stem Cells (MSCs)

MSCs may confer additional benefits because of its immunosuppressive advantages (29-30). A seven-day survival was recorded after the mouse bone marrow stem cells were placed into the gerbil cochlea (scala tympani or modiolus) (31). Jeon et al. showed that the combination of growth factors and the over-expression of Math1 could induce the differentiation of bone marrow MSCs into the hair cells. The co-culture of neurosensory progenitors with the developing sensory epithelium cells ,even in the absence of Math1 expression, can result in sensory cells (32).

Neural Stem Cells

In order to differentiate the auditory neurons, neural stem cells were delivered into inner ear (14, 33-37). In 2012 ,Chen et al. studied an embryonic stem cell derived from otic progenitors, and their results showed the restoration of auditory evoked responses (38). Other neural stem cells may be more applicable for SGN (Spiral Ganglion Neuron) replacement (11, 31, 39-40). Iguchi et al. reported a 10% survival (for 25 days) a glial phenotype(GFAP&Nestin immunostaining), in the replacement of neural stem cells from fetal mouse neuroepithelium into the mouse cochlea (35). Results indicated that cisplatin treatment followed by the implantation of mouse neural stem cells into modiolus of the mouse cochleae, the majority of cells differentiated into a glial phenotype (GFAP immunostaining), and few cells could reach a neuronal phenotype (36). Hu et al. implanted neural stem cells from the lateral wall of mouse lateral ventricle into the normal or deafened guinea pig cochlea. After III β-tubulin and GFAP immunostaining, they have found a few neuronal and many glial phenotype respectively (34). moderate levels of survival have been found with differentiation into satellite cell, SGN, Schwann cell, hair cell and supporting cell-like phenotypes (39).

Growth and neurotropic factors in inner ear tissue engineering

One of the most effective signaling in the inner ear development is IGF (41). IGF-1 is effective in

prevention of hair cells damage by noise and ototoxic drugs in vivo and in vitro, respectively (42-43). Activation of both PI3K/Akt and MEK/ERK pathways by IGF-1 can lead to the maintenance of hair cells in injured cochlea (44). The differentiation or survival of neurons neurotrophic factors are of importance. Furthermore, some of these factors play an important role in normal development and protection of hair cell damage in non-neuronal tissues such as inner ear. Neurotrophin priority in crystal canal is Brain-derived neurotrophic factor (BDNF) and the expression of neurotrophin-3 (NT-3) is limited and delayed (45). In in vivo protection of drug-induced damage of inner ear and development of auditory sensory epithelium (46), BDNF and NT-3 were most effective respectively. Both BDNF and NT-3 were found to be effective against ototoxic drug in rat auditory hair cells (47). Loss of BDNF and NT-3 can reflect the differential expression level during the development. In vestibular system, loss of both have distinct effects, while their loss in cochlea may be largely quantitative (48). Because of neurotrophin expression in hair cells and supporting cells, even if sensory epithelia never differentiate, by manipulating of neurotrophins could lead to retain long-term innervation for better use of cochlear implants in cases of progressive hair cell loss. Several studies reported that postnatal expression of neurotrophins is basically different from what is reported by in situ hybridization (45). The coexpression of BDNF and NT-3 in delaminating neurons is one of the strongest arguments regarding which all neurons are derived from the ear (49). The expression of neurotrophins prior to the differentiation of the hair cells, suggest that some neurotrophin expressions are independent of hair cell differentiation (50). Some cells in the organ of Corti (OC) provide neurotrophin factors,in which their loss is considered as one of the most significant causes of SGN degeneration. In order to protect SGNs from degeneration, we can use exogenous neurotrophins, however, the effects will remain as long as there is the source of NTs. Cochlear implantation requires SGN survival, therefore, adjuvant therapy for enhancing SGN performance might be considered in patients with severe-profound hearing loss. Direct infusion of NT-3 and BDNF into cochlea via an osmotic mini-pump, could remarkably enhance SGN survival, however, it is considered a short-term solution and continuous infusion is impossible. Since one strategy to provide a cellular source of NTs, is neurotrophin gene transfer into the cells within cochlea, It can be considered as a long-term solution for SGN survival after hair cell loss (51). In the deaf cochlea with a normal innervate of OC, we can use residual OC cells for neurotrophin gene transfer. However this method is limited in preventing SGN loss when the cells are continuously destroyed in the organ of Corti (52).

Biomaterials and Scaffolds used in inner ear tissue engineering

Polymer scaffolds have many different functions in the field of tissue engineering. They are applied as space filling agents, as delivery vehicles for bioactive molecules, and as three-dimensional structures that organize cells and present stimuli to direct the formation of a desired tissue. Several hydrogel systems currently exist in which proteins are successfully incorporated into a scaffold and then released (53). In a study, they demonstrated an efficient transfer of BDNF into inner ear using a biodegradable hydrogel. This indicates that the

placement of a biodegradable hydrogel on RWM is an effective method for the local application of neurotrophins to the inner ear. In the present study, ELISA analyses confirmed sustained delivery of BDNF to cochlear fluid during 7 days using a biodegradable hydrogel. In addition, functional and morphological protection of SGNs was observed 7 days after BDNF application, indicating that its biological effects were maintained during this period (54).

Polymer biomaterials are used in the treatment of hearing disorders such as; hereditary deformity in the bone tissue of the ear, ear pain and ear swelling and middle ear inflammation. Porous polyethylene is used to replace incus, stapes and malleus, which are obtained by implant stabilization through bone growth in porosity. Polytetrafluoroethylene and silicon for the repair of ear injuries, and polytetrafluoroethylene-carbon and pyrolytic polyethylene-carbon composites for inner ear cochlea replacement were being evaluated. Unlike hearing aids, these implants do not sound louder or clearer, but directly stimulate the auditory nerves. Many research have been conducted regarding polymers such as polypyrrole, as an electrode in hearing implants (55).

Use of decellularized tissues has become popular in tissue engineering applications as the natural extracellular matrix can provide necessary physical cues that help induce the restoration and development of functional tissues. In the study of decellularized cochlear tissue was used as a scaffold. The role of the scaffold is support the exogenous cells (56).

References

1. Staudenmaier R, Arnold W. Aesthetics and functionality in ear reconstruction: Karger; 2010.
2. Staudenmaier R, Mandlik V, Schurr C, Burghartz M, Hauber K, Meier G, et al. Customized tissue engineering for ear reconstruction. Aesthetics and Functionality in Ear Reconstruction: Karger Publishers; 2010. p. 120-31.
3. Sterodimas A, de Faria J, Correa WE, Pitanguy I. Tissue engineering and auricular reconstruction: a review. Journal of Plastic, Reconstructive & Aesthetic Surgery. 2009;62(4):447-52.
4. Senn P, Heller S. [Stem-cell-based approaches for treating inner ear diseases]. Hno. 2008;56(1):21-6.
5. Pauley S, Kopecky B, Beisel K, Soukup G, Fritzsch B. Stem cells and molecular strategies to restore hearing. Panminerva medica. 2008;50(1):41.
6. Martinez-Monedero R, Edge AS. Stem cells for the replacement of inner ear neurons and hair cells. International Journal of Developmental Biology. 2007;51(6/7):655.
7. Ahn K, Jeon S, Jung J, Kim Y, Kang J. Isolation of embryonic stem cells from enhanced green fluorescent protein-transgenic mouse and their survival in the cochlea after allotransplantation. Cytotherapy. 2008;10(7):759-69.
8. Altschuler RA, O'Shea KS, Miller JM. Stem cell transplantation for auditory nerve replacement. Hearing research. 2008;242(1):110-6.

9. Coleman B, Hardman J, Coco A, Epp S, De Silva M, Crook J, et al. Fate of embryonic stem cells transplanted into the deafened mammalian cochlea. Cell transplantation. 2006;15(5):369.
10. Hu Z, Ulfendahl M, Olivius NP. Central migration of neuronal tissue and embryonic stem cells following transplantation along the adult auditory nerve. Brain research. 2004;1026(1):68-73.
11. Lang H, Schulte BA, Goddard JC, Hedrick M, Schulte JB, Wei L, et al. Transplantation of mouse embryonic stem cells into the cochlea of an auditory-neuropathy animal model: effects of timing after injury. Journal of the Association for Research in Otolaryngology. 2008;9(2):225-40.
12. Okano T, Nakagawa T, Endo T, Kim T-S, Kita T, Tamura T, et al. Engraftment of embryonic stem cell-derived neurons into the cochlear modiolus. Neuroreport. 2005;16(17):1919-22.
13. Praetorius M, Vicario I, Schimmang T. Efficient transfer of embryonic stem cells into the cochlea via a non-invasive vestibular route. Acta oto-laryngologica. 2008;128(7):720-3.
14. Regala C, Duan M, Zou J, Salminen M, Olivius P. Xenografted fetal dorsal root ganglion, embryonic stem cell and adult neural stem cell survival following implantation into the adult vestibulocochlear nerve. Experimental neurology. 2005;193(2):326-33.
15. Reyes JH, O'Shea KS, Wys NL, Velkey JM, Prieskorn DM, Wesolowski K, et al. Glutamatergic neuronal differentiation of mouse embryonic stem cells after transient expression of neurogenin 1 and treatment with BDNF and GDNF: in vitro and in vivo studies. The Journal of Neuroscience. 2008;28(48):12622-31.
16. Sekiya T, Kojima K, Matsumoto M, Kim T-S, Tamura T, Ito J. Cell transplantation to the auditory nerve and cochlear duct. Experimental neurology. 2006;198(1):12-24.
17. Shi F, Corrales CE, Liberman MC, Edge AS. BMP4 induction of sensory neurons from human embryonic stem cells and reinnervation of sensory epithelium. European Journal of Neuroscience. 2007;26(11):3016-23.
18. Corrales CE, Pan L, Li H, Liberman MC, Heller S, Edge AS. Engraftment and differentiation of embryonic stem cell–derived neural progenitor cells in the cochlear nerve trunk: Growth of processes into the organ of corti. Journal of neurobiology. 2006;66(13):1489-500.
19. Ulfendahl M, Hu Z, Olivius P, Duan M, Wei D. A cell therapy approach to substitute neural elements in the inner ear. Physiology & behavior. 2007;92(1):75-9.
20. Nakagawa T, Ito J. Cell therapy for inner ear diseases. Current pharmaceutical design. 2005;11(9):1203-7.
21. Oshima K, Suchert S, Blevins NH, Heller S. Curing hearing loss: Patient expectations, health care practitioners, and basic science. Journal of communication disorders. 2010;43(4):311-8.
22. Oshima K, Shin K, Diensthuber M, Peng AW, Ricci AJ, Heller S. Mechanosensitive hair cell-like cells from embryonic and

induced pluripotent stem cells. cell. 2010;141(4):704-16.

23. Martinez-Monedero R, Edge AS. Stem cells for the replacement of inner ear neurons and hair cells. International Journal of Developmental Biology. 2007;51(6-7):655-61.

24. Rivolta MN, Li H, Heller S. Generation of inner ear cell types from embryonic stem cells. Embryonic Stem Cell Protocols: Volume 2: Differentiation Models. 2006:71-92.

25. De Coppi P, Bartsch G, Siddiqui MM, Xu T, Santos CC, Perin L, et al. Isolation of amniotic stem cell lines with potential for therapy. Nature biotechnology. 2007;25(1):100-6.

26. Donaldson AE, Cai J, Yang M, Iacovitti L. Human amniotic fluid stem cells do not differentiate into dopamine neurons in vitro or after transplantation in vivo. Stem cells and development. 2009;18(7):1003-12.

27. Prusa A-R, Marton E, Rosner M, Bettelheim D, Lubec G, Pollack A, et al. Neurogenic cells in human amniotic fluid. American journal of obstetrics and gynecology. 2004;191(1):309-14.

28. Zong L, Chen K, Zhou W, Jiang D, Sun L, Zhang X, et al. Inner ear stem cells derived feeder layer promote directional differentiation of amniotic fluid stem cells into functional neurons. Hearing research. 2014;316:57-64.

29. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nature Reviews Immunology. 2008;8(9):726-36.

30. Ohtaki H, Ylostalo JH, Foraker JE, Robinson AP, Reger RL, Shioda S, et al. Stem/progenitor cells from bone marrow decrease neuronal death in global ischemia by modulation of inflammatory/immune responses. Proceedings of the National Academy of Sciences. 2008;105(38):14638-43.

31. Matsuoka AJ, Kondo T, Miyamoto RT, Hashino E. In Vivo and In Vitro Characterization of Bone Marrow-Derived Stem Cells in the Cochlea. The Laryngoscope. 2006;116(8):1363-7.

32. Jeon S-J, Oshima K, Heller S, Edge AS. Bone marrow mesenchymal stem cells are progenitors in vitro for inner ear hair cells. Molecular and Cellular Neuroscience. 2007;34(1):59-68.

33. Fu Y, Wang S, Liu Y, Wang J, Wang G, Chen Q, et al. Study on neural stem cell transplantation into natural rat cochlea via round window. American journal of otolaryngology. 2009;30(1):8-16.

34. Hu Z, Wei D, Johansson CB, Holmström N, Duan M, Frisén J, et al. Survival and neural differentiation of adult neural stem cells transplanted into the mature inner ear. Experimental cell research. 2005;302(1):40-7.

35. Iguchi F, Nakagawa T, Tateya I, Kim TS, Endo T, Taniguchi Z, et al. Trophic support of mouse inner ear by neural stem cell transplantation. Neuroreport. 2003;14(1):77-80.

36. Tamura T, Nakagawa T, Iguchi F, Tateya I, Endo T, Kim T, et al. Transplantation of neural stem cells into the modiolus of mouse

cochleae injured by cisplatin. ACTA OTOLARYNGOLOGICA SUPPLEMENTUM. 2004:65-8.

37. Tateya I, Nakagawa T, Iguchi F, Kim TS, Endo T, Yamada S, et al. Fate of neural stem cells grafted into injured inner ears of mice. Neuroreport. 2003;14(13):1677-81.
38. Chen W, Jongkamonwiwat N, Abbas L, Eshtan SJ, Johnson SL, Kuhn S, et al. Restoration of auditory evoked responses by human ES-cell-derived otic progenitors. Nature. 2012;490(7419):278-82.
39. Parker MA, Corliss DA, Gray B, Anderson JK, Bobbin RP, Snyder EY, et al. Neural stem cells injected into the sound-damaged cochlea migrate throughout the cochlea and express markers of hair cells, supporting cells, and spiral ganglion cells. Hearing research. 2007;232(1):29-43.
40. Wang Y, Dong M. In vitro induction and differentiation of newborn guinea pig hippocampus neural stem cells into cells resembling inner hair cells, using artificial perilymph. The Journal of Laryngology & Otology. 2011;125(08):771-5.
41. Okano T, Xuan S, Kelley MW. Insulin-like growth factor signaling regulates the timing of sensory cell differentiation in the mouse cochlea. Journal of Neuroscience. 2011;31(49):18104-18.
42. Lee KY, Nakagawa T, Okano T, Hori R, Ono K, Tabata Y, et al. Novel therapy for hearing loss: delivery of insulin-like growth factor 1 to the cochlea using gelatin hydrogel. Otology & Neurotology. 2007;28(7):976-81.
43. Angunsri N, Taura A, Nakagawa T, Hayashi Y, Kitajiri S-i, Omi E, et al. Insulin-like growth factor 1 protects vestibular hair cells from aminoglycosides. Neuroreport. 2011;22(1):38-43.
44. Hayashi Y, Yamamoto N, Nakagawa T, Ito J. Insulin-like growth factor 1 inhibits hair cell apoptosis and promotes the cell cycle of supporting cells by activating different downstream cascades after pharmacological hair cell injury in neonatal mice. Molecular and Cellular Neuroscience. 2013;56:29-38.
45. Fariñas I, Jones KR, Tessarollo L, Vigers AJ, Huang E, Kirstein M, et al. Spatial shaping of cochlear innervation by temporally regulated neurotrophin expression. Journal of Neuroscience. 2001;21(16):6170-80.
46. Lidian A, Stenkvist–Asplund M, Linder B, Anniko M, Nordang L. Early hearing protection by brain-derived neurotrophic factor. Acta oto-laryngologica. 2012;133(1):12-21.
47. Ruan RS, Leong SK, Mark I, Yeoh KH. Effects of BDNF and NT-3 on hair cell survival in guinea pig cochlea damaged by kanamycin treatment. Neuroreport. 1999;10(10):2067-71.
48. Yang T, Kersigo J, Jahan I, Pan N, Fritzsch B. The molecular basis of making spiral ganglion neurons and connecting them to hair cells of the organ of Corti. Hearing research. 2011;278(1):21-33.
49. Rubel EW, Fritzsch B. Auditory system development: primary auditory neurons and their targets. Annual review of neuroscience. 2002;25(1):51-101.

50. Jahan I, Pan N, Kersigo J, Fritzsch B. Beyond generalized hair cells: molecular cues for hair cell types. Hearing research. 2013;297:30-41.
51. Kesser B, Lalwani A. Gene therapy and stem cell transplantation: strategies for hearing restoration. Gene Therapy of Cochlear Deafness: Karger Publishers; 2009. p. 64-86.
52. Wise AK, Tu T, Atkinson PJ, Flynn BO, Sgro BE, Hume C, et al. The effect of deafness duration on neurotrophin gene therapy for spiral ganglion neuron protection. Hearing research. 2011;278(1):69-76.
53. Drury JL, Mooney DJ. Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials. 2003;24(24):4337-51.
54. Endo T, Nakagawa T, Kita T, Iguchi F, Kim TS, Tamura T, et al. Novel strategy for treatment of inner ears using a biodegradable gel. The Laryngoscope. 2005;115(11):2016-20.
55. Peterson DR, Bronzino JD. Biomechanics: principles and applications: CRC press; 2007.
56. Mellott AJ, Shinogle HE, Nelson-Brantley JG, Detamore MS, Staecker H. Exploiting decellularized cochleae as scaffolds for inner ear tissue engineering. Stem cell research & therapy. 2017;8(1):41.