Smart Materials in Medicine 2 (2021) 350–353

Contents lists available at ScienceDirect

Smart Materials in Medicine

journal homepage: www.keaipublishing.com/en/journals/smart-materials-in-medicine/

Perspective

Nanoparticle-based inner ear delivery systems for the treatment of hearing loss

Xiaoyu Xu [a,b], Shengyi Wang [a], Yilai Shu [a,c,**], Hongbo Zhang [b,d,*]

[a] ENT Institute and Department of Otorhinolaryngology, Eye & ENT Hospital, State Key Laboratory of Medical Neurobiology, Institutes of Biomedical Sciences, Fudan University, Shanghai, 200031, China
[b] Pharmaceutical Sciences Laboratory, Faculty of Science and Engineering, Åbo Akademi University, Turku, 20520, Finland
[c] NHC Key Laboratory of Hearing Medicine, Fudan University, Shanghai, 200031, China
[d] Turku Bioscience Centre, University of Turku and Åbo Akademi University, Turku, 20520, Finland

ARTICLE INFO

Keywords:
Hearing loss treatment
Nanoparticles
Round window membrane penetration
Targeting delivery
Stimuli-responsive

ABSTRACT

Hearing loss has become the most common sensory disorder worldwide. Despite intensive research on the pathophysiology of hearing loss, biological therapeutic approaches are limited by the anatomical and physiological characteristics of the inner ear. Challenges in inner ear drug delivery involve biotherapeutic instability, membrane inaccessibility and delivery non-specificity. With the development of nanotechnology, the nanoparticle-based delivery systems are promising to overcome these limitations. After drugs loading and stabilization, nanoparticles can carry the drugs into the inner ear by crossing round window membrane. The surface bioconjugation of ligand endows nanoparticles with specific targeting capability. Nanoparticles with stimuli-responsive moieties can provide a controlled release manner. Together, these strategies show great potential for hearing loss treatment. Understanding the current advances of nanotechnology in hearing loss treatment will facilitate future therapeutic options and clinical applications.

According to the latest data from the World Health Organization (WHO), more than 466 million people worldwide suffer from hearing loss, of which 34 million are children and 432 million are adults, and the number is expected to rise to 2.5 billion by 2050[1]. Although hearing aids and cochlear implants offer treatment options somehow, they do not restore the underlying pathology and require invasive surgery. At the pre-clinical level, there is much evidence for hearing disorders treatment through the delivery of medications to inner ear[2]. The anatomy of ear is illustrated in Fig. 1. The inner ear is almost surrounded by the hardest bone in the body and has separate compartments. Hence, the therapeutical options for inner ear are narrow due to its poor accessibility and extreme vulnerability. The current methods, including systemic circulation, intratympanic injection and direct inner ear transport, are often accompanied with challenges of efficiency, safety and invasiveness[3]. Both the physiological and anatomical issues along with low long-term stability of drugs set significant barriers to drug penetration, resulting in low drug concentration at the desired sites[4]. In order to overcome the problems of conventional drug administration in inner ear, novel delivery approaches must be explored to provide a noninvasive, targeted and controllable carrier for drug transport.

In recent years, the delivery of therapeutic molecules with nanoparticles has been considered as a promising method to restore hearing functions because of their various advantages, including small size, high loading capacity and surface functionalization[5]. The customization of nanoparticles has led to their application in non-invasive drug delivery, stabilization, specific targeting, and controlled release. Many nanoparticle-based carriers have been constructed for the inner ear drug delivery, such as lipids, polymers, inorganic nanoparticles. Fig. 2 demonstrates the designed nanocarriers for hearing loss treatment.

Parallel to the development of delivery systems, the nanocarrier administrations have also been studied. Drug delivery through the cochlear round window membrane (RWM), the connection between the inner and middle ear, seems working as the dominant route[5]. The nanoparticles that can diffuse through the RWM offer the possibility of introducing drugs into the cochlea without causing surgical trauma to the sensitive

* Corresponding author. Pharmaceutical Sciences Laboratory, Faculty of Science and Engineering, Åbo Akademi University, Turku, 20520, Finland.
** Corresponding author. ENT Institute and Department of Otorhinolaryngology, Eye & ENT Hospital, State Key Laboratory of Medical Neurobiology, Institutes of Biomedical Sciences, Fudan University, Shanghai, 200031, China.
E-mail addresses: yilai_shu@fudan.edu.cn (Y. Shu), hongbo.zhang@abo.fi (H. Zhang).

https://doi.org/10.1016/j.smaim.2021.10.002
Received 19 September 2021; Received in revised form 5 October 2021; Accepted 6 October 2021
Available online 2 November 2021

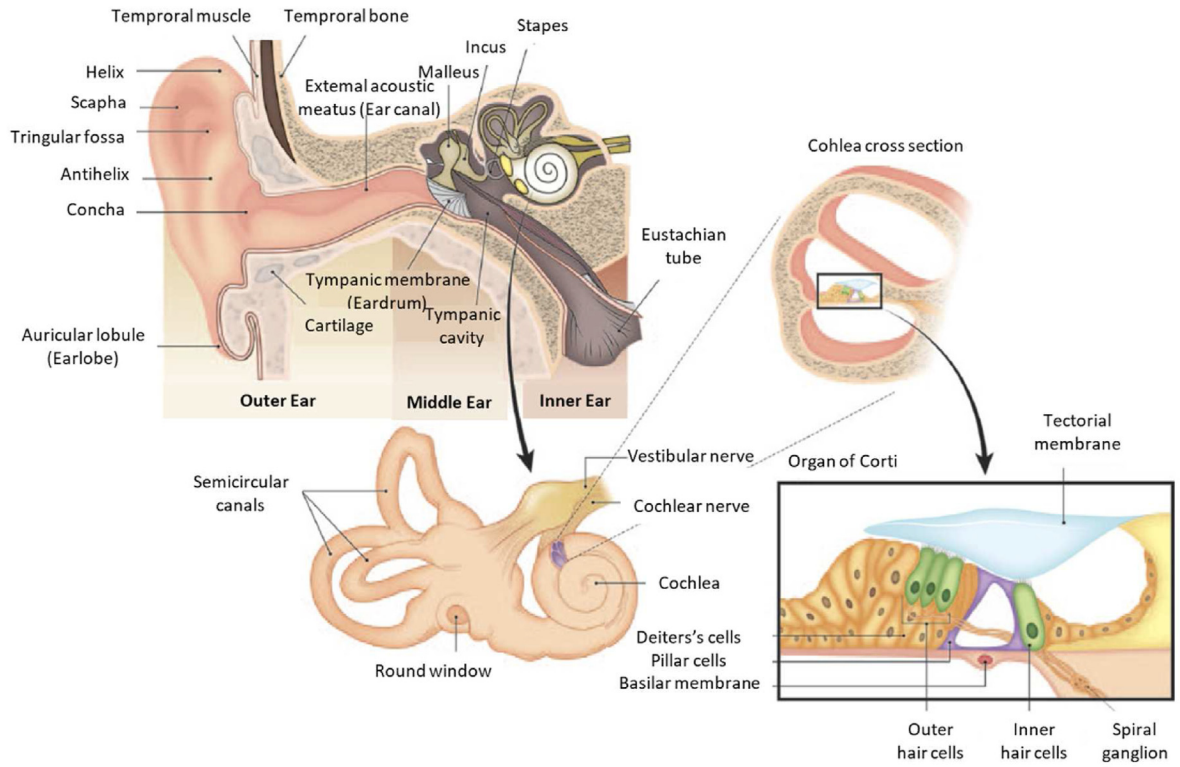

Fig. 1. The anatomical structure of ear and the detailed structure of inner ear. Adapted with permission from [2]. Under the license of CC BY-NC.

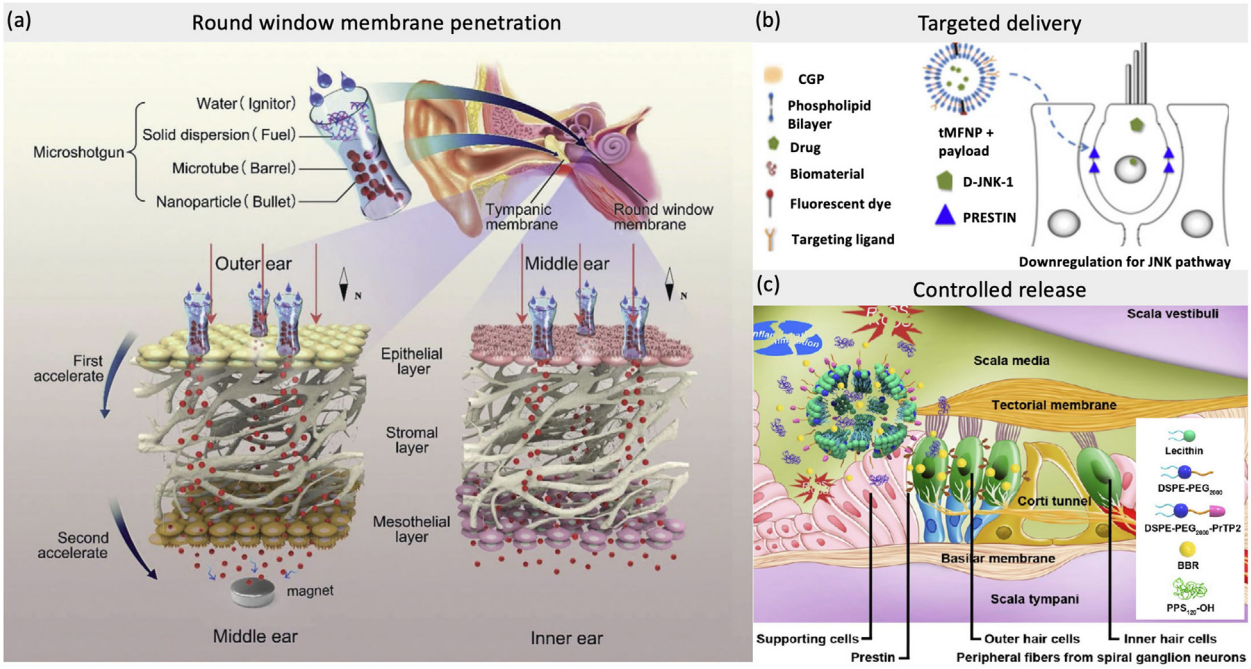

Fig. 2. Nanoparticle-based delivery system for hearing loss treatment: (a) A microshotgun delivery system for the non-invasive *trans*-tympanic and *trans*-RWM delivery of magnetic nanoparticles. Adapted with permission from [12]. Copyright (2020), Elsevier. (b) Targeted nanohydrogel delivery system to OHCs. Adapted with permission from [14]. Copyright (2018), Elsevier. (c) ROS-responsive nanoparticle for berberine delivery. Adapted with permission from [15]. Copyright (2021), American Chemical Society.

inner ear. In addition to the membrane thickness of the RWM, factors such as size, charge, conformation and lipid solubility have a strong influence on its permeability[6]. Smaller particles are more readily transported through the RWM, for example, Zou and coworkers prepared three sizes of liposome nanoparticles (95, 130 and 240 nm) and explored their distribution after *trans*-tympanic injection[7]. The results show that 95 nm particles have the most effective transport behavior, whereas the 240 nm particles were transported least. Usually, nanoparticles with size smaller than 200 nm can effectively penetrate the RWM in *in vivo* models [8]. Regarding the charge, previous studies have shown that the

administration of positively charged nanoparticles own high permeability of RWM, which might be caused by their highly intracellular uptake efficiency[9]. In addition, attempts have been made to increase the RWM permeability by surface modification. Nanoparticles that modified with cell-penetrating peptides (CPPs), especially low molecular weight protamine (LMWP) showed a prominent RWM penetration[10]. In this study, they also observed that nanoparticles at 150 nm and 300 nm had a faster cochlear entry rate than that nanoparticles at 80 nm. Therefore, the unique properties of nanoparticle can affect the speed of entry. Besides the RWM penetration in animal models, nanoparticles were also shown their RWM crossing ability in freshly frozen human temporal bone. The crossing mechanism is initially believed to occur via the paracellular pathway by diffusion of intracellular pathway[11]. Compared to the passive diffusion, the penetration of nanoparticles through RWM under physical means exhibit greater ability. Liang et al. successfully prepared an inner ear delivery system, termed as microshotgun (MS) (Fig. 2a)[12]. The MS was composed by microtube, Fe_3O_4 nanoparticles and solid dispersion, which utilized as the barrel, bullets, and fuel of MS, respectively. The tympanic membrane (TM) and RWM transport was designed by a rocket two-stage acceleration theory, where the solid dispersion (organic acids and sodium bicarbonate) helped the nanoparticles to cross the epithelial layer and magnetic force guide them through the innermost layer. This prepared MS showed greatly improved transmembrane efficiency with low toxicity to the cells or tissues of the ear. Other external forces, including light, electronic and ultrasonic can also be used for this propose. But before that, more efforts are needs to evaluate the toxicity and injury to the cells or tissues caused by these forces.

After administration, nanoparticle-based carriers have demonstrated their capability for targeting delivery in the cochlea via surface modification. Bioconjugation has been proved as a useful method for ligands anchoring to the nanoparticle by the stable peptide bonds. Until now, various nanocarriers have been achieved for auditory hair cells and spiral ganglion neurons (SGNs). The outer hair cells (OHCs) have one specifically expressed electromotility protein, prestin[4]. The modification of prestin-targeted ligands to nanoparticles has increased their specific binding to OHCs, leading to better therapeutic effect. For instance, a peptide (termed A666) with specific binding affinity for prestin was modified to maleimide poly(ethylene glycol)-poly(lactide) nanoparticles by maleimide-thiol coupling technique[13]. Following administration, most of A666 modified nanoparticles targeted to the OHCs and only a few of them were taken up by other cell types, while the nanoparticles without A666 did not home to the OHCs. As a result, dexamethasone loaded A666-nanoparticles exhibited the significant protecting efficiency against cisplatin-induced hearing loss (CIHL) in guinea pigs. Recently, a new peptide, prestin-targeting peptide 1 (PrTP1), that can recognize some extracellular domines of prestin was used to construct a targeted nanohydrogel delivery system (Fig. 2b)[14]. This system showed higher uptake efficiency *in vitro* and higher delivery capability to the apical region *in vivo* compared to non-targeted systems. After incorporating the c-Jun *N*-terminal kinase (JNK) inhibitor, D-JNKi-1, into the delivery system, the payload was successfully transported to OHCs and promoted protection against noise-induced hearing loss (NIHL). By the improvement of the basis of PrTP1, prestin-targeting peptide 2 (PrTP2) was designed and modified to berberine (BBR) nanocarrier for OHC-targeted treatment of NIHL[15]. Besides, the ability of nanoparticles to target to the spiral ganglion cells (SGCs) and cochlear nerve (CN) has been demonstrated, which creates an advanced therapeutic strategy for sensorineural hearing loss treatment. Approved by US Food and Drug Administration, poly(Ɛ-caprolactone)-block-poly(ethylene glycol) (PEG-b-PCL) polymersomes (PMs) can entry SGCs efficiently. Targetability of PMs to the CN was achieved by Tet peptide (binding to the trsialoganglioside clostridial toxin receptors in in neuronal membranes) functionalization through cochleostomy injection[16]. In a similar approach, targeting peptide, came from human nerve growth factor (hNGF) beta, was modified onto the terminal of hydrophilic part of PMs [17]. Due to the receptor-mediated endocytosis, hNGF-tegged PMs shown a high selective-uptake level in sensory hair cells, nerve cells and the nerve fibers. By comparation, the nonspecific uptake in control group was obtained in a low level. The ligands anchoring to nanocarriers indeed improve the therapeutic effect while increasing the systemic complexity. More suitable *in vitro* and *in vivo* models of auditory system need to be employed to determine their biocompatibility.

In addition, stimuli-responsive delivery systems based on nanoparticles have been investigated for hearing loss therapy. These nanocarriers are useful for the on-demand delivery of therapeutical molecules with minimized doses. NIHL comes with inflammation and overproduction of reactive oxygen species (ROS). Recently, a ROS-responsive nanoparticle was developed and utilized as BBR carriers for NIHL therapy[15]. As shown in Fig. 2c, lecithin, PPS120–OH, DSPE-PEG2000, PrTP2-modified DSPE-PEG2000 and BBR self-assembled into PL-PPS/BBR. After penetrating the inner ear by RWM and accumulating around OHC areas, the PPS120 in PL-PPS/BBR occurred structural transformation in ROS environment to releasing BBR rapidly. As a result, the morphological integrity of OHCs were protected and hearing in NIHL guinea pig was improved significantly. Own to the high glycolysis rate and high lactic acid concentration, the pH in inflamed tissues (5.5–6.8) is lower than that in normal tissues. Roman et al. prepared one pH-responsive polymer nanoparticles with antioxidant and anti-inflammatory characters toward CIHL[18]. The system was created by the reaction of amphiphilic bioactive copolymers that were methacrylic derivatives of α-tocopheryl succinate and ibuprofen. After dexamethasone loading, the nanoparticle formulation shown reduced CDDP-induced toxicity *in vitro* and hearing loss from CDDP in rats. Considering the less available of intrinsic stimuli in inner ear, external stimulus can be introduced. After the accumulation of nanoparticles in the targeted area, the release behavior is activated by light, ultrasound, or other triggers at desire time. Generally, the external stimulus is more spatiotemporal and has higher potential for clinical applications.

To summarize, nanoparticle-based delivery system offers a promising tool for non-invasive and efficient treatment of hearing loss. Biomaterials, including liposome, polymer and inorganic nanoparticles can across the RWM into the inner ear by adjusting of particle size, charge, surface modification and physical power. Surface bioconjugation of ligand to nanoparticles allows targeting delivery to specific inner ear cells. In addition, nanoparticles with stimuli-responsive moieties provide controlled release of the loaded cargoes. Although successful entry of nanocarriers into the inner ear has been reported in animal models, the useful information about human ototoxicity, potential organ side-effect and long-term impact on ear health of nanoparticles are lacking. Hence, future studies on nanoparticle-mediated therapy must take these aspects into consideration. The development of biocompatible and multifunctional nanoparticles that can delivery drugs in targeted, controlled and safety manner should be a promising perspective. Currently, there has been an increase in the number of ongoing clinical trials utilizing nanoparticles for hearing loss treatment. A preclinical phase using poly(lactic-co-glycolic acid) carrying lidocaine for inner ear application has been conducted. Sametime, the application of IGF-1 with gelatin hydrogel on the RWM was performed in Phase I/IIa and shown hearing improvement in 56% of patients with sensorineural hearing loss. While clinical trails related nanoparticles are primarily polymeric materials, there is a trend towards the development of more complex nanoparticles. The first and most important issue is the biocompatibility of new and emerging nanocarriers. Future clinical approaches might take advantage of the modularity of nanoparticles to implement specifically targeting subsets of inner ear cells. Of course, this is based on the discovery of more specific receptors on inner ear cells. It is believed that he integration and cross of multidiscipline, including chemistry, biology and clinical medicine can provide better technical support for nanoparticle-based therapeutic options, leading to more efficient and more specific treatment of hearing loss.

CRediT authorship contribution statement

Xiaoyu Xu: Writing – original draft. **Shengyi Wang:** Visualization. **Yilai Shu:** Conceptualization, Writing – review & editing. **Hongbo Zhang:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work is supported by Academy of Finland (Grant No. 328933), Finland China Food and Health International Pilot Project funded by the Finnish Ministry of Education and Culture (No. 280M0052K1) and Sigrid Jusélius Foundation (decision No. 28002247 K1). This work is also part of the activities within the strategic research profiling area Solutions for Health at Åbo Akademi University (Academy of Finland, # 336355).

References

[1] Deafness and hearing loss. https://www.who.int/news-room/fact-sheets/detail/deafness-and-hearing-loss, 2021.
[2] J.H. Lee, M.Y. Lee, Y. Lim, J. Knowles, H.W. Kim, Auditory disorders and future therapies with delivery systems, J. Tissue Eng. 9 (2018).
[3] D.C. Gheorghe, A.G. Niculescu, A.C. Birca, A.M. Grumezescu, Nanoparticles for the treatment of inner ear infections, Nanomaterials-Basel 11 (2021).
[4] L.L. Li, T. Chao, J. Brant, B. O'Malley, A. Tsourkas, D.Q. Li, Advances in nano-based inner ear delivery systems for the treatment of sensorineural hearing loss, Adv. Drug Deliv. Rev. 108 (2017) 2–12.
[5] R. Mittal, S.A. Pena, A. Zhu, N. Eshraghi, A. Fesharaki, E.J. Horesh, J. Mittal, A.A. Eshraghi, Nanoparticle-based drug delivery in the inner ear: current challenges, limitations and opportunities, Artif. Cell Nanomed. B 47 (2019) 1312–1320.
[6] B. Szeto, H. Chiang, C. Valentini, M. Yu, J.W. Kysar, A.K. Lalwani, Inner ear delivery: challenges and opportunities, Laryngoscope Invest 5 (2020) 122–131.
[7] D.K. Kim, Nanomedicine for inner ear diseases: a review of recent in vivo studies, BioMed Res. Int. 2017 (2017).
[8] S. Martin-Saldana, R. Palao-Suay, M.R. Aguilar, R. Ramirez-Camacho, J.S. Roman, Polymeric nanoparticles loaded with dexamethasone or alpha-tocopheryl succinate to prevent cisplatin-induced ototoxicity, Acta Biomater. 53 (2017) 199–210.
[9] J. Chester, E. Johnston, D. Walker, M. Jones, C.M. Ionescu, S.R. Wagle, B. Kovacevic, D. Brown, M. Mikov, A. Mooranian, H. Al-Salami, A review on recent advancement on age-related hearing loss: the applications of nanotechnology, Drug Pharmacology, and Biotechnology, Pharmaceutics 13 (2021).
[10] H. Cai, Z.P. Liang, W.L. Huang, L. Wen, G. Chen, Engineering PLGA nano-based systems through understanding the influence of nanoparticle properties and cell-penetrating peptides for cochlear drug delivery, Int. J. Pharm. 532 (2017) 55–65.
[11] S. Nyberg, N.J. Abbott, X. Shi, P.S. Steyger, A. Dabdoub, Delivery of therapeutics to the inner ear: the challenge of the blood-labyrinth barrier, Sci. Transl. Med. 11 (2019), eaao0935.
[12] Z.P. Liang, H. Yu, J.F. Lai, L. Wen, G. Chen, An easy-to-prepare microshotgun for efficient transmembrane delivery by powering nanoparticles, J. Contr. Release 321 (2020) 119–131.
[13] X.L. Wang, Y.M. Chen, Y. Tao, Y.G. Gao, D.H. Yu, H. Wu, A666-conjugated nanoparticles target prestin of outer hair cells preventing cisplatin-induced hearing loss, Int. J. Nanomed. 13 (2018) 7517–7531.
[14] M.N. Kayyali, J.R.A. Wooltorton, A.J. Ramsey, M. Lin, T.N. Chao, A. Tsourkas, B.W. O'Malley, D.Q. Li, A novel nanoparticle delivery system for targeted therapy of noise-induced hearing loss, J. Contr. Release 279 (2018) 243–250.
[15] Z.Q. Zhao, Z.Z. Han, K. Naveena, G.X. Lei, S.W. Qiu, X.Y. Li, T. Li, X. Shi, W. Zhuang, Y.L. Li, Y.H. Qiao, H.M. Liu, ROS-responsive nanoparticle as a berberine carrier for OHC-targeted therapy of noise-induced hearing loss, Acs Appl. Mater. Inter. 13 (2021) 7102–7114.
[16] Y. Zhang, W.K. Zhang, A.H. Johnston, T.A. Newman, I. Pyykko, J. Zou, Targeted delivery of Tet1 peptide functionalized polymersomes to the rat cochlear nerve, Int. J. Nanomed. 7 (2012) 1015–1022.
[17] S. Roy, A.H. Johnston, T.A. Newman, R. Glueckert, J. Dudas, M. Bitsche, E. Corbacella, G. Rieger, A. Martini, A. Schrott-Fischer, Cell-specific targeting in the mouse inner ear using nanoparticles conjugated with a neurotrophin-derived peptide ligand: potential tool for drug delivery, Int. J. Pharm. 390 (2010) 214–224.
[18] S. Martin-Saldana, R. Palao-Suay, M.R. Aguilar, L. Garcia-Fernandez, H. Arevalo, A. Trinidad, R. Ramirez-Camacho, J. San Roman, pH-sensitive polymeric nanoparticles with antioxidant and anti-inflammatory properties against cisplatin-induced hearing loss, J. Contr. Release 270 (2018) 53–64.