Review

Auditory disorders and future therapies with delivery systems

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Abstract

Auditory function takes a major part in human life. While sensorineural hearing loss is related with many factors including genetic disorders, age and noise, the clear causes are not well understood. Even more, the currently available treatments with drugs cause side effects, which thus are considered suboptimal. Here, we communicate the delivery systems with biomaterials that can be possible therapeutic options to restore hearing and vestibular functions. We introduce briefly the various pathological factors related with hearing loss and the limitation of current therapies, detail the recent studies on delivery systems including nanoparticles and hydrogels and discuss future clinical availability.

Keywords

Hearing loss, nanoparticle, hydrogel, inner ear delivery, clinical availability

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Ear structure and sensorineural hearing losses

In this part, the structure of ear and the common causes of hearing loss are briefly described, and this can help further discussion on the therapies and strategies to be developed for the treatment of auditory disorders.

Figure 1 depicts the ear anatomical structure. It is macroscopically divided into three parts (external, middle and inner ear), and the inner ear is composed of the cochlea and vestibule that play an important role in hearing and balance. When soundwave moves through the canal of the outer and middle ear and hits the tympanic membrane, force is transmitted into oval window connected with scala tympani in the cochlea. This interaction causes physiological transduction between the tectorial membranes and hair cells in the organ of Corti amplify the signal transmission to the brain.

Two important sensory cells, namely, inner hair cells (IHCs) and outer hair cells (OHCs) that are located in the core part of the ear, are responsible for hearing function. While the IHCs are organized into one layer and transmit the electrophysiological stimulus into the brain via the cochlear nerve, the OHCs are organized into three layers and amplify soundwaves. The electrical signal is transduced to spiral ganglion cells that are innervated to the auditory nerve. The other cells (Deiters' and pillar cells) also attach to the hair call layers supporting them. Of note, these sensory cells (OHCs and IHCs) do not regenerate once damaged in mammals,[1] making therapeutic treatment of sensorineural hearing loss utmost difficult.

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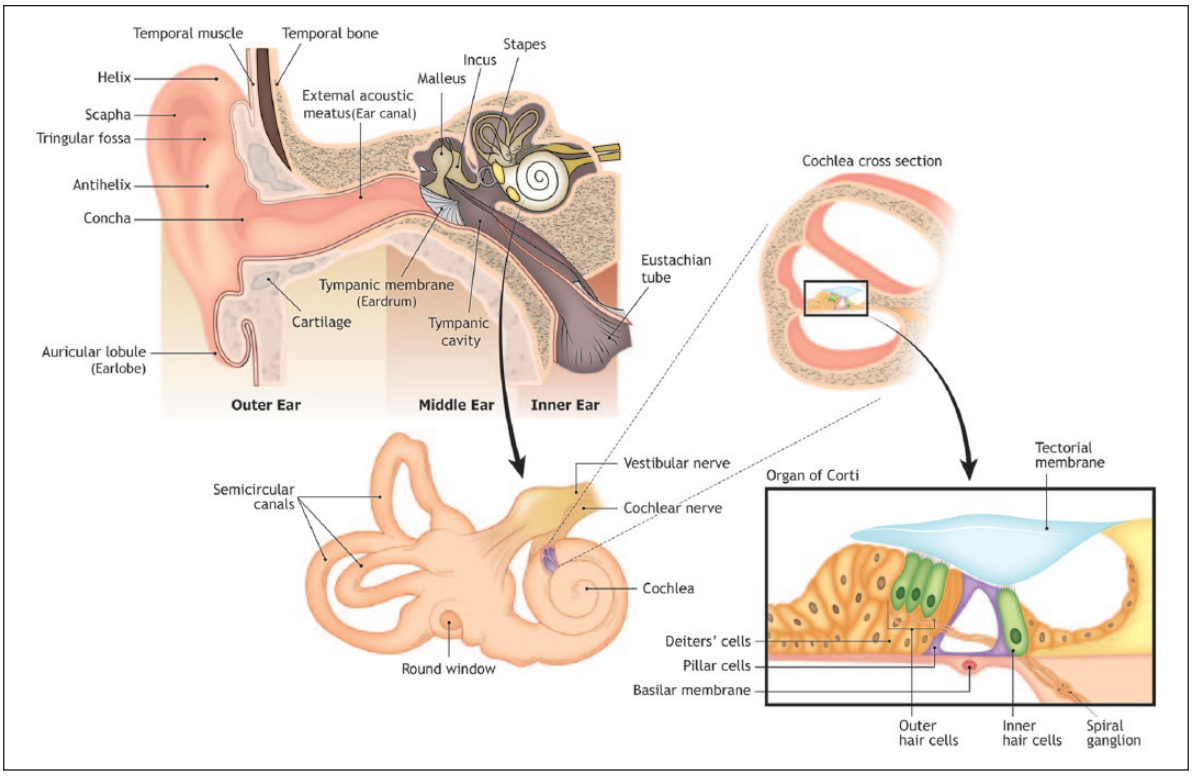

Figure 1. Anatomy of ear and the structure of organ of Corti.

It is known that several factors, including social factors, heredity and pharmacological side effects cause hearing impairment, and many hearing disorders (approximately 37%) are caused by social factors, such as life-related noise and age.[2] Disease in the external and middle ear also results in conductive hearing loss, which is mostly reversible and can be treated by medication, surgery and devices to augment acoustic stimuli. However, the anatomical complexity of the inner ear and the limited regenerative functions, the treatment of cochlear (sensorineural) hearing loss via drug or surgery is challenging. Among others, noise-induced hearing loss (NIHL) is the most common life-related noise-induced disease which is defined as an occupational disease due to exposure to extreme noise in the workplace. Intense noise can result in auditory damage and injury to hair cells in the inner ear.[3] Most NIHL is caused by physical damage to hair cells by pathological mechanical stimuli via fluid vibrations in the cochlea. In the United States, the prevalence of NIHL among noise-exposed workers is high: 23% with hearing loss, 15% with tinnitus and 9% with both disorders.[4] In addition, many factors, such as systemic conditions (i.e. high blood pressure and diabetes) contribute to age-related hearing loss, making it difficult to distinguish the causes.

Genetic links are ranked as the second most prevalent factor, responsible for approximately 20% of the hearing-impaired population.[5] The majority of genetic hearing loss is non-syndromic; therefore, an initial diagnosis without a screening protocol to investigate genetic mutations is difficult. Among the discovered hearing loss–related genes, connexin-related gene mutations that are responsible for cell-to-cell communication are the most common.[6,7] In addition, some phenotypes are accompanied by syndromic hearing losses, such as Usher[8–11] and Pendred syndrome.[12–15]

Several pharmacologic agents, such as aminoglycoside and cisplatin can also cause sensorineural hearing loss.[16,17] These drugs trigger the pathological production of reactive oxygen species in hair cells in the inner ear and lead to hair cell apoptosis after uptake from the peri/endo-lymph during systemic blood circulation. Preventive approaches for hearing loss induced by these drugs are highly required but remain under investigation because there are currently no definite solutions to address this issue.

Current treatments and limitations

Some current treatments with drugs or implantable devices are clinically available for auditory dysfunctions. For a long time (over 60 years), the corticosteroid therapy has been employed for particularly for a small portion of sensorineural hearing loss and acute cases such as sudden hearing loss, Meniére's disease and immune-mediated hearing loss.[18] However, there is no medical therapy available for cases of 'chronic' sensorineural hearing loss, which albeit are more predominant than acute cases. The

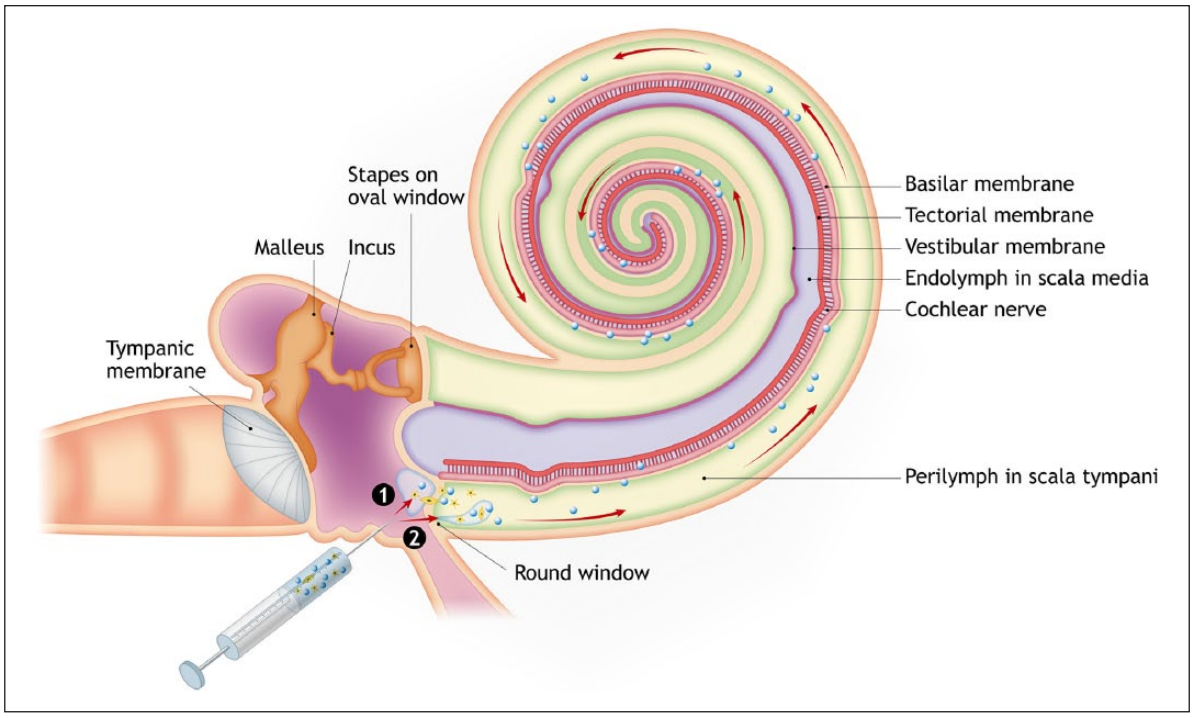

Figure 2. Round window niche in inner ear serves as a delivery route for the treatment of auditory diseases. Biomaterials (hydrogels or nanoparticles) can be injected around (1) or through (2) the round window membrane (RWM) to deliver therapeutic molecules or cells to inner ear space.

only way relies on rehabilitation using amplification devices and cochlear implants, which remain suboptimal in terms of the direct recovery of hair cells and improvement of hearing functions. Therefore, some recent studies have attempted to find better solutions for the treatment of chronic sensorineural hearing loss using biomaterials and stem cells.

On the other hand, hearing aid – a small electronic device that can be worn in or behind the ear – amplifies sound vibrations, thus helping people hear better. Hair cells in the inner ear are able to detect better the increased vibrations, converting them effectively into neural signals to brain. However, there is a practical limitation to the amplification level that can be provided by hearing aid.[19] In addition, if the inner ear is severely damaged, even the high vibration cannot be transmitted into neural signals, making the hearing aid ineffective in this situation. Cochlear implant is a different form of hearing aid currently applicable in clinical settings.[20] While the hearing aid amplifies sounds such that they can be detected by a damaged ear, the cochlear implant bypasses the damaged portions of the ear and directly stimulates the auditory nerve. Signals generated by the implant are sent via the auditory nerve to the brain, which recognizes the signals as sound. Cochlear implants have to fulfil a number of requirements, including mechanical stability, the ability to transfer charge to the auditory nerve, biocompatibility and long-term stability. Therefore, cochlear implants are generally composed of silicone, titanium, platinum and ceramics.[21] Many clinical studies have shown the effectiveness of cochlear implants for patients with hearing loss due to noise or genetic disorders; however, some hurdles still remain; suboptimal restoration of hearing function, availability only for hearing spoken language (not music), relatively long training period (1–2 years) and high cost.[22] In addition, those hearing devices, including hearing aids and cochlear implants, are less accepted for cosmetic reasons.

Delivery systems for hearing disorders

For the systemic delivery, high doses of drugs are required to targeted areas of poor blood circulation, such as the inner ear, which however, leads to unexpected severe adverse effects in other parts of the body. Therefore, the approach of local injection into the middle ear is preferred. For this, the delivery of drugs is through the oval window or round window membrane (RWM). In particular, the RWM is a unique channel to the cochlea or vestibular tissue, but it consists of a few layers of epithelial membranes (~100 μm) thus is considered a significant barrier for drug penetration[23] (Figure 2). Often a small diameter needle is used in order not to cause anatomical disruption. When a molecule or particle enters the inner ear organ, it experiences the stream of a fluid moving at a speed of a few μm/s, traversing the inner ear circulation for approximately

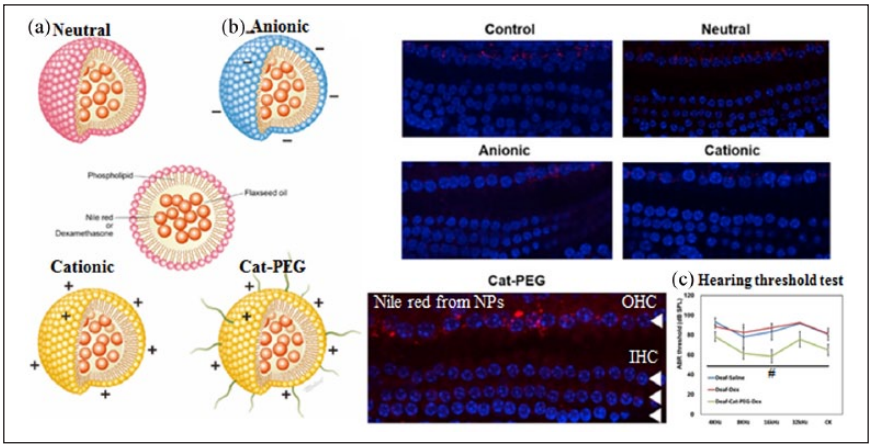

Figure 3. Optimized phospholipid-based nanoparticles for inner ear drug delivery and therapy. (a) Schematic diagram of four candidate nanoparticles obtained from phospholipid nanoemulsions and loaded with Nile red for tracking or dexamethasone for therapy and (b) their in vivo distribution in the organ of Corti of the inner ear. The intensity of the Nile red fluorescence (red) absorbed around the outer hair cells (OHCs, blue-stained nucleus) or inner hair cells (IHCs, blue-stained nucleus) of the organ of Corti obtained with Cat-PEG nanoparticles was significantly higher than that obtained with the other nanoparticles. (c) Therapeutic outcomes afforded by dexamethasone (Dex)-loaded Cat-PEG in a mouse model of ototoxicity. The Deaf-Cat-PEG-Dex group exhibited significantly better hearing at all frequencies tested than the Deaf-DexP and Deaf-saline control groups (#, $p = 0.05$, $n = 6$). Adopted from Yang et al.[34]

2 h before gradually escaping to the lymphatic vessel.[24] Therefore, an optimal carrier system should be able to address the following two issues: (1) penetrating the epithelial layers on the cochlear surface and (2) targeting cells of interest, such as IHCs, OHCs and spiral ganglion cells, while in the circulating perilymph.

Among other candidates, NPs have attracted significant attention in inner ear research due to their various advantages, including small size, injectability, loading capacity and ability to undergo diverse chemical modifications. Furthermore, other injectable forms of biomaterials (e.g. hydrogels) have also been studied. This section summarizes the delivery systems that have been developed for the treatment of inner ear disorders.

Nanoparticles

The delivery of therapeutic molecules with NPs is considered a promising approach for restoring hearing function. NPs can encapsulate various therapeutic agents (drugs, proteins or genes) and deliver them to target cells and even cellular organelles.[25,26] After penetrating RWM, the NPs can reach hair cells (IHCs and OHCs) in the cochlea.

Among the delivery carriers, polymeric, magnetic, hydroxyapatite, silica NPs,[27] liposomes and polymersomes[28] have been studied for the treatment of the inner ear through penetration of the RWM.[29] Biocompatible and degradable biopolymers were initially investigated, and the surface modification of the NPs was shown to be effective for overcoming the barriers (epithelial membrane penetration and cellular uptake) to inner ear delivery.[30] Among other surface modifications, polyethylene glycol (PEG) coating, namely, PEGylation, is regarded as a promising method due to the increased diffusivity into cells or tissues.[31] It was demonstrated that fluorescent dye-tagged NPs that were PEGylated exhibited significantly higher fluorescence levels in OHCs of the organ of Corti compared with those without PEGylation.[32] As an applicable example of drug delivery, PEG-coated polylactic acid (PEG-PLA) NPs loaded with dexamethasone were locally injected on the surface of the RWM[33] to promote survival of the hair cells in the presence of cisplatin-induced ototoxicity and the maintenance of auditory function in guinea pigs.

Another recent finding is that the charge of NPs can determine their uptake in hair cells and their epithelial membrane penetration. The role of the charge of phospholipid-based NPs was investigated by preparing NPs of comparable nanoparticle size (180~280 nm) with almost neutral (–4 mV), negative (–26 mV), or positive (+ 26 mV) charge or PEGylated (0 mV) NPs. It was revealed that positively charged NPs were intracellularly taken up by hair cells at an approximately two-fold higher rate than neutrally and negatively charged NPs due to the electrical interaction between the positive charge of NPs and the negative charge of the outer lipid layer. A similar investigation using artificial mouse penetration as the first barrier showed that almost neutral or PEGylated NPs exhibited higher rates of intracellular delivery compared with the other groups, indicating the determinant role of a neutral charge in tackling two structural barriers, namely, epithelial layers and the cellular membrane (Figure 3).[34]

The targeting strategies developed thus far have focussed on the precise delivery to a specific type of cell or

intracellular organ. Conventional cell-targeting peptides, such as the transactivator of transcription (TAT) peptide (for overcoming the lipophilic barrier) and the nuclear localization sequence (NLS) (for delivering cargos to the nucleus), can be tagged to NPs to enhance the efficiency of delivery.[35] OHC-targeting peptide, a composition of transmembrane protein (prestin) expressed exclusively in OHCs, was recently successfully conjugated to NPs to allow the targeted delivery of cargos of interest to OHCs.[36] In addition, neurotrophin receptors (NTRs), tropomyosin-related kinase receptor tyrosine kinase (Trk) and p75 NTR are specifically expressed on hair cells and spiral ganglion neurons in the inner ear. Thus, NPs designed to target specific receptors (TrkB receptors and p75 NTR) by tagging with ligands could lead to increased therapeutic efficiency by selectively delivering to target cells.[37–39]

The value of the abovementioned targeting systems is their widespread application in other delivery systems, including hydrogels and scaffolds. In addition, the targeting release strategy can be applied to the glutathione-, pH- or laser-responding release of cargos from NPs.[40] These stimuli-responsive delivery systems are useful for the on-demand delivery of drug molecules while minimizing the doses required, which can potentiate the therapeutic efficacy of the drugs in many inner ear disorders.

Various compositions of NPs have been investigated for inner ear delivery. Among them, liposomes, which are composed of the same material as the cell membrane, are the most commonly used carriers due to their commercial availability, efficient epithelial penetration efficiency and the easy modification of their surface hydrophilicity and charge.[41] For example, liposomes (size of 240 nm) containing magnetic resonance imaging (MRI) tracking dye were intratympanically injected onto the middle-inner ear barriers (oval window and RWM), and the uptake of liposomes in the inner ear was observed using a rat model.[42] Chitosan, a linear polymer consisting of randomly distributed D-glucosamine and N-acetyl-D-glucosamine, is another NP source applied to the inner ear delivery systems due to its biocompatibility and high positive charge, which are helpful characteristics for penetrating the lipid cell membrane.[43,44] The intracellular uptake efficiency of low-molecular-weight chitosan is equivalent to that of polyethylenimine (PEI), which is one of the most efficient non-viral biomolecules for penetrating cell membranes.[45] Mesoporous silica nanoparticles (MSNs) are widely used in delivery systems due to their high porosity, high loading capacity, visualization ability (e.g., carbon dots), excellent biocompatibility and easy surface modification. However, the delivery efficacy to inner ear organs has not been well investigated and thus requires further studies of inner ear delivery.[46–48] Polymersomes consisting of amphiphilic block copolymers are vesicular, nano-sized spheres that encapsulate an aqueous solution and lead to the delivery of large amounts of biomolecules of interest. Because polymersomes are easily modified by chemical conjugation and tagging peptides to target a specific site, promising results, including for OHCs, have been reported.[49,50]

While the efficiency of epithelial membrane penetration and cellular uptake should be considered in the design of NPs for inner ear delivery, the detection of NPs is also important. When injected, the NPs penetrate the RWM and possibly reach hair cells; thus, the distribution of NPs in the organ of Corti and vestibular tissue indicates the efficacy of the delivery system. In many cases, the direct incorporation of fluorescent dyes is used for optical detection, but other imaging methods (e.g. CT, MRI and ultrasound) can also be used if the NPs incorporate proper detection dyes or carbon dots in their innate structure. For example, MRI-traceable liposome NPs were developed by encapsulating gadolinium-tetra-azacyclododecane-tetra-acetic acid (Gd-DOTA), which is an MRI tracing dye.[42]

Although there has been significant progress in the development of NPs for delivery systems, their use for the treatment of auditory functions has been relatively less explored. Given the merits of the localized organ properties of the auditory system, which would allow the more efficient delivery of the injected NPs to target cells, utilizing a wealth of strategies for NPs designed for other purposes (e.g. cancer treatment) can advance the efficacy of NP-based drug delivery systems for inner ear diseases, which warrants further study.[51,52]

Hydrogels

Hydrogels are soft materials networked by physically or chemically crosslinked biopolymers in aqueous solutions.[53] In general, they can hold large amounts of water and thereby incorporate large amounts of biomolecules.[54] In addition, the water inside the hydrogels allows the diffusion of loaded biomolecules, and their release pattern can be controlled by tailoring the polymer networks. Some hydrogels exhibit a unique property, called stimuli-responsive property, and thereby undergo an abrupt change in physical properties (i.e. volume, stiffness, degradability and shape) in response to micro-environmental changes, including pH, temperature and enzymes (i.e. matrix metalloproteinases) produced by cells they are in contact with.[55–57] Therefore, an increasing number of approaches have used hydrogels for the delivery of biomolecules to the inner ear by their injection into the middle ear, which causes the biomolecules to diffuse to the inner ear through epithelial membranes (as depicted in Figure 2).

Some in vivo and even clinical studies have proven the efficacy of hydrogels in delivering therapeutic molecules for inner ear treatment. As an example, hydrogel was used for glucocorticoid delivery. In general, the systemic uptake of glucocorticoid has been recommended as a standard therapy for sudden hearing loss. However, the recovery rate of patients can be as low as 20%,[58] which is mainly

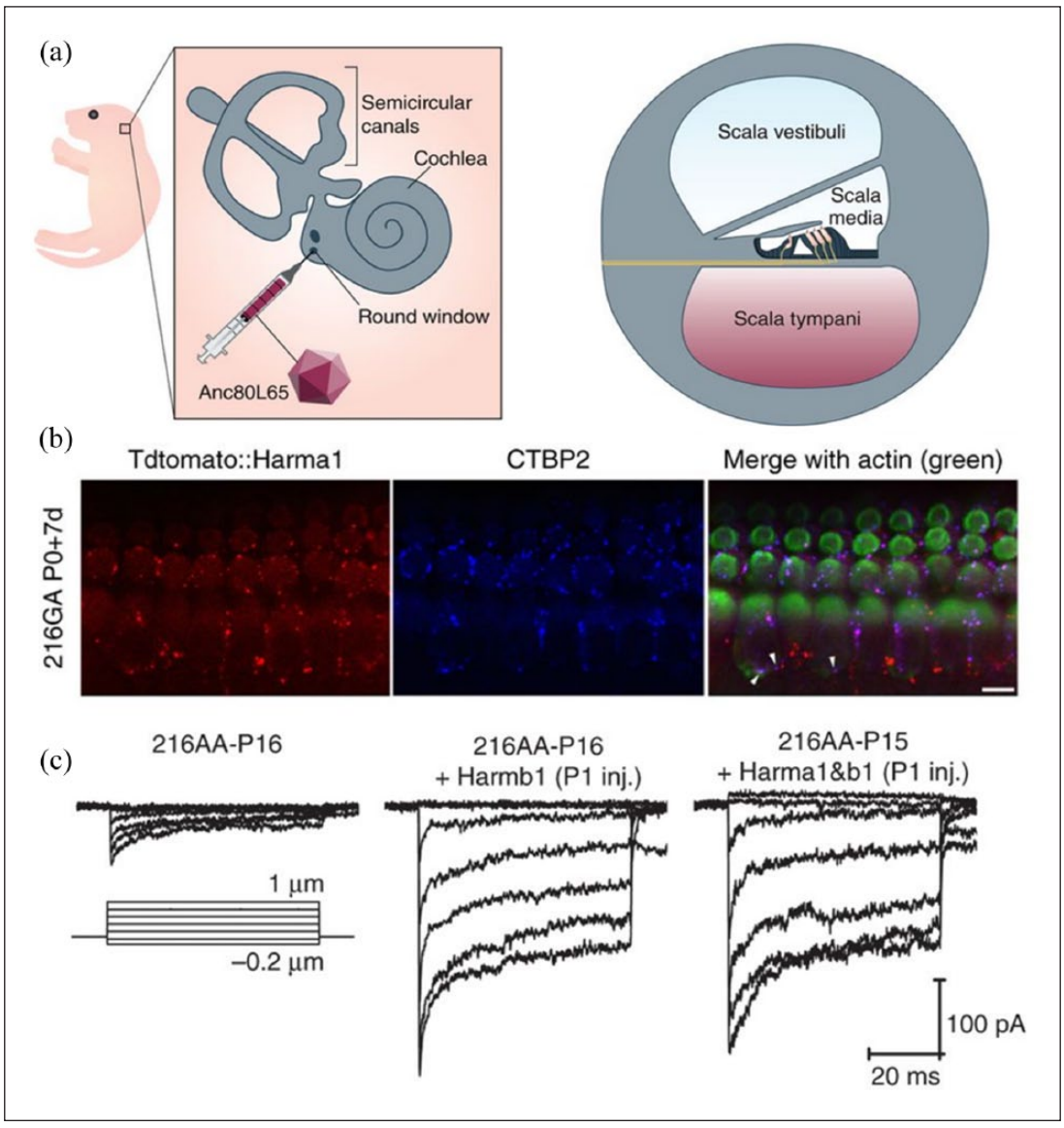

Figure 4. Hearing rescue by the gene delivery approach: a synthetic virus-incorporated harmonin gene (AAV2/Anc80L65.CMV. harmonin-b1) to the inner ear of Usher syndrome type IC mice with mutated harmonin gene (216AA (homozygous) or 216GA (heterozygous)). (a) Schematic image of the injection site and the injected virus associated with the transgene (Anc80L65) inside the scala tympani in the cochlea, which allows it to gain access to structurally and functionally immature neonatal hair cells. (b) Exogenous tdTomato-harmonin-a1 (red) was detected in the cell body of hair cells (CTBP2, blue) in P7 organotypic cultures exposed to AAV2/1.CMV.tdTomato-harmonin-a1 for 24 h at P0 (scale bar, 5 μm). (c) Recovery of the mechanotransduction current in hair cells of mice injected with Anc80L65 harmonin vectors (Harmb1 or Harma1&b1). Adopted from Pan et al.[11]

due to the low efficiency of the targeting in the drugs to hair cells. Therefore, the use of hydrogels has been investigated as an alternative approach for the efficient delivery of drugs.[59] A clinical study placed gelatine hydrogels impregnated with recombinant human IGF1 containing 300 μg of mecasermin in the RWM. The patients who received this treatment showed some hearing improvement after 12 weeks. As other examples, PLGA-PEG-PLGA hydrogels were used for sustained drug release in guinea pigs through intratympanic injection,[60] and this approach was applied in the clinical setting to locally deliver glucocorticoids for hearing recovery in patients with sudden sensorineural hearing loss resistant to systemic treatment.[61] In addition, hydrogels are used as a matrix for drug/biomolecule delivery in cochlear implants. Chikar et al.[62] used dual PEDOT- and RGD-functionalized alginate hydrogel coatings to achieve sustained drug delivery. The poly (3,4-thylenedioxythiophene) (PEDOT) coating reduced the electrode impedance and shifted the phase angle in vitro, *and* BDNF was released from the hydrogel coating into the cochlea. Cochlear implants are operated by electrically stimulating the auditory nerves to enhance

auditory function. Better outcomes were obtained when the implants were coated with arg-gly-asp (RGD)-functionalized alginate hydrogel and conducting polymer poly (3,4-ethylenedioxythiophene).

Gene delivery systems

Genetic disorders are also major causes of the hearing loss. Therefore, gene delivery to the inner ear organ to inhibit hearing loss has been a focus of otology research.[63] Viral vectors are generally investigated to rescue or protect auditory and vestibular disorders. For examples, adeno-associated viral vectors, such as AAV1, 2, 6, 8, and Anc80L65, have shown greater transfection efficiency in inner ear delivery.[11,64] One recent study aimed to restore the complex auditory and balance functions by the Ush1c gene delivery to mice with Usher syndrome (Figure 4). The synthetic Anc80L65 vectors showed notable efficiency in transducing the Ush1c gene in up to 90% of sensory hair cells, leading to restoration of the complex auditory and balance behaviour to near wild-type levels.[11] AAV2/8 vectors that encode wild-type whirlin restored IHCs, but the auditory function and OHCs were not restored.[65] In another study, AAV2/1 vectors were injected into Tmc1-mutant mice, and this gene therapy restored a moderate level of hearing function with a minimal auditory-brainstem-response threshold.[66] A similar viral capsid and a promoter that restricted expression to IHCs partially restored auditory function in mice deficient in the IHC gene Vglut3.[67] Furthermore, the cellular tropism of a novel adeno-associated bovine virus vector (BAAV) was used for efficient transduction of the inner ear without pathological effects, and the number of transduced hair cells in the cochlea and vestibular tissue was increased with BAAV.[68]

Even with some of the potential effects of gene therapy, the utilization of viral vectors is not considered clinically relevant, except in some limited cancer research,[69,70] due to possible tumorigenesis and unexpected adverse effects from virus integration in human DNA. Therefore, non-viral delivery systems using NPs might be an alternative that has not yet been utilized in clinical settings. Non-viral NP delivery systems can encounter the following potential barriers in the auditory system: (1) the gene carriers should overcome the RWM mucosa barrier, (2) after penetrating the RWM, gene carriers should specifically navigate to the target cells, such as IHCs and OHCs, and (3) gene carries need to penetrate the cell membrane and release/deliver genetic molecules to the nucleus while escaping lysosomal degradation. During this long journey filled with several barriers, a large number of carriers can be lost, and the activity of the genes can also be decreased; therefore, future studies should investigate the optimal design of nanocarriers for gene delivery. The optimal physical characteristics (size and charge) obtained with chemical surface modification and specific ligand tagging to target cell types and the cell nucleus should be considered when designing NPs for gene therapy systems.

Concluding remarks

Ear converts soundwave into the brain through the nervous vestibulocochlearis, and the fluid movement in vestibular organ contributes to balance perception. Therefore, ear is considered an important sensory organ in social life and maintaining body safety while perceiving various environmental signals. As discussed, several factors, such as life-related noise, age, idiopathic causes and genetic disorders, are involved in hearing impairment. Although prophylaxis and drug administration therapies via oral uptake or intravenous injection have been clinically available for the treatment of hearing impairment, some adverse effects are still encountered with high doses of drugs. Also, for the case of ear syndromes and disorders, there is no clinical options available. Therefore, new strategies to the delivery of therapeutic molecules to the inner ear are highly demanded.

Recent experimental studies have highlighted the active role of biomaterials for the treatment of ear disorders. Direct injection of therapeutic drugs with biomaterials into middle ear is considered one of the best options for inner ear delivery. Among else, NPs and hydrogels offer promising platforms for the efficient loading and controlled delivery of therapeutic molecules with much reduced side effects. Furthermore, those delivery systems can overcome several anatomical barriers to reach the target cells in the inner ear, including the penetration of epithelial layer and target cell membrane, and the escape of lysosomal degradation inside cells. In the future therapy, non-viral gene delivery with NPs is also considered for the treatment of ear disorders caused by genetic syndromes due to their safety.

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