Nanoscale Horizons

ROYAL SOCIETY OF CHEMISTRY

REVIEW

View Article Online
View Journal | View Issue

Cite this: *Nanoscale Horiz.*, 2025, **10**, 2641

Nanotechnology-enhanced gene therapy for hearing loss

Yiwen Liu,[ab] Lin Li,[b] Pei Huang,[c] Dingjun Zha*[a] and Hongzhang Deng*[b]

Hearing loss is a global health concern affecting hundreds of millions of individuals, with current interventions like hearing aids and cochlear implants offering only functional improvements rather than addressing the root causes. Gene therapy holds promise for restoring hearing by correcting underlying molecular defects, but its success hinges on the safe and effective delivery of therapeutic genes to the delicate cells of the cochlea. Nanotechnology has emerged as a transformative solution, providing non-viral carriers such as nanoparticles that can protect genetic material, target specific inner ear cells, and promote regeneration with minimal invasiveness. This review explores the integration of nanotechnology with gene therapy for hearing loss from three perspectives: (1) the biological mechanisms underlying hearing loss and gene therapy, (2) technological innovations in nanoparticle design and delivery systems, and (3) clinical applications and challenges. By examining recent advancements and preclinical studies, this review highlights the potential of nanotechnology-enhanced gene therapy to revolutionize the treatment of hearing loss while addressing key barriers to clinical translation.

Received 22nd July 2025,
Accepted 29th July 2025

DOI: 10.1039/d5nh00520e

rsc.li/nanoscale-horizons

1. Introduction

Hearing loss is a widespread sensory impairment that affects over 460 million people worldwide, with significant impacts on quality of life, communication, and social integration.[1–8] Current interventions, such as hearing aids and cochlear implants, provide functional improvements but do not address the underlying molecular or cellular causes of hearing loss.[9–12] In contrast, gene therapy offers the potential to restore hearing by correcting genetic mutations, modulating gene expression, or promoting the regeneration of damaged cells within the cochlea.[13–16] However, the delivery of therapeutic genes to the inner ear remains a significant challenge due to the cochlea's delicate structure, the presence of the blood-labyrinth barrier, and the limited regenerative capacity of cochlear cells.[13,17–23]

Recent advancements in gene therapy have shown potential in addressing genetic forms of hearing loss.[23–27] For instance, mutations in the OTOF gene, which encodes the protein otoferlin essential for synaptic transmission in inner hair cells, have been identified as a cause of hereditary hearing loss.[7,16,28–31] Patients with OTOF mutations often experience severe to profound hearing loss and speech impairments. Gene therapy approaches aim to introduce functional copies of the OTOF gene into affected cells to restore hearing function.[32–34] Experimental gene therapies, such as DB-OTO and AAVAnc80-OTOF, are being developed to address otoferlin-related hearing loss.[35,36] These therapies utilize adeno-associated virus (AAV) vectors to deliver the OTOF gene to target cells in the inner ear. Preclinical studies have demonstrated that dual AAV vector delivery can restore otoferlin expression and improve hearing in animal models.[37–40]

Nanotechnology has emerged as a powerful tool to overcome the challenges associated with gene delivery to the inner ear.[41–45] Nanoparticles, composed of biocompatible materials such as lipids, polymers, or inorganic compounds, can be engineered to enhance specificity, reduce immunogenicity, and improve the durability of gene expression.[46–50] For example, polymer-based nanoparticles have been investigated for their potential to deliver therapeutic genes directly to the inner ear.[41,51] These nanoparticles can encapsulate genetic material, protecting it from degradation and ensuring targeted delivery to specific cochlear cells. Studies have demonstrated that polymer-based nanoparticles can effectively deliver genes to inner ear cells, highlighting their potential in gene therapy applications.[41,51–53] Additionally, lipid-based nanoparticles have been explored for their ability to traverse the blood-labyrinth barrier and deliver therapeutic agents to the inner ear.[54–56] Their biocompatibility and capacity to encapsulate various therapeutic molecules make them suitable candidates for gene therapy

[a] *Department of Otolaryngology-Head and Neck Surgery, Xijing Hospital, Air Force Military, Xi'an, Shaanxi 710032, China. E-mail: zhadjun@fmmu.edu.cn*
[b] *Engineering Research Center of Molecular & Neuroimaging, Ministry of Education School of Life Science and Technology, Xidian University, Xi'an, Shaanxi 710126, China. E-mail: hzdeng@xidian.edu.cn*
[c] *Departments of Diagnostic Radiology, Surgery, Chemical and Biomolecular Engineering, and Biomedical Engineering, Yong Loo Lin School of Medicine and Faculty of Engineering, National University of Singapore, Singapore 119074, Singapore*

applications. Advancements in nanoparticle surface modification have enabled the development of targeted delivery systems that recognize and bind to specific receptors on cochlear cells, enhancing the precision and efficacy of gene delivery.

Despite these advancements, several challenges remain in the clinical application of nanotechnology-enhanced gene therapy for hearing loss. Ensuring the long-term safety and efficacy of these therapies is paramount, necessitating extensive preclinical and clinical evaluations. Addressing potential immunogenic responses to nanoparticle-based delivery systems is also crucial for successful translation into clinical settings.[57–59] Furthermore, optimizing the delivery methods to achieve precise targeting and efficient gene transfer to the appropriate cochlear cells remains a significant challenge.

The integration of nanotechnology and gene therapy holds transformative potential for treating hearing loss. Technological innovations in nanoparticle design and delivery systems have paved the way for targeted and efficient gene therapy approaches. Understanding the biological mechanisms underlying hearing loss has facilitated the development of gene therapies aimed at correcting genetic mutations and promoting hair cell regeneration. While challenges remain in translating these therapies to clinical practice, ongoing research and clinical trials continue to advance the field, offering hope for effective treatments that can restore hearing and improve the quality of life for individuals affected by hearing loss. This review provides a comprehensive examination of the intersection between nanotechnology and gene therapy for hearing loss, structured around three key themes: (1) the technological innovations in nanoparticle design and delivery systems, (2) the biological mechanisms underlying hearing loss and the potential of gene therapy to address them, and (3) the clinical applications, challenges, and future directions for this promising therapeutic approach. By synthesizing recent advancements and preclinical findings, this review aims to highlight the transformative potential of nanotechnology-enhanced gene therapy in restoring hearing and improving the lives of individuals with hearing loss.

1.1. Biological mechanisms of hearing loss and advances in gene therapy

Hearing loss arises from various underlying mechanisms that disrupt the normal function of the auditory system. Broadly, it can be classified into sensorineural, conductive, and mixed hearing loss, each with distinct causes and pathophysiological effects.[2,3,60,61] Sensorineural hearing loss (SNHL) is primarily linked to damage in the cochlea or auditory nerve, often due to genetic mutations, aging, or external factors such as noise exposure and ototoxic substances.[62,63] Conductive hearing loss (CHL), in contrast, results from mechanical obstructions or dysfunctions in the outer or middle ear, impeding the transmission of sound waves to the inner ear.[20,64–66] Some individuals experience mixed hearing loss, a combination of both conditions, where conductive impairments coexist with sensorineural deficits.

Beyond these classifications, hearing loss can also be syndromic or non-syndromic, with syndromic forms associated with broader systemic disorders affecting multiple organs.[67] Additionally, the onset of hearing loss can be congenital or acquired, with congenital cases often resulting from genetic factors or prenatal infections, while acquired cases stem from environmental exposures, aging, or trauma. Understanding these biological mechanisms is crucial for developing targeted therapies, including pharmacological treatments, prosthetic devices, and emerging gene therapy interventions that aim to repair or regenerate auditory structures at the molecular level.

As current treatments, such as hearing aids and cochlear implants, primarily compensate for hearing deficits rather than restoring auditory function, gene therapy has emerged as a promising approach to address the root causes of hearing impairment.

1.1.1. Sensorineural hearing loss (SNHL). Sensorineural hearing loss (SNHL) occurs due to damage or dysfunction in the cochlear hair cells, the auditory nerve, or their associated structures within the inner ear.[68–70] This type of hearing loss is often permanent and can result from genetic mutations, aging, noise exposure, ototoxic drugs, or infections[71] (Fig. 1). In genetic cases, mutations in critical genes such as GJB2 (connexin 26), MYO7A (myosin VIIA), and TMC1 disrupt essential auditory processes, leading to progressive or congenital deafness. Acquired causes, such as noise-induced damage or ototoxicity from aminoglycoside antibiotics and chemotherapy drugs, can result in irreversible cochlear cell death.

The pathophysiology of SNHL involves several mechanisms, including hair cell degeneration, synaptic dysfunction, and auditory nerve impairment.[72–74] Age-related hearing loss, or presbycusis, arises from oxidative stress and mitochondrial damage, leading to a gradual decline in hair cell and neural function. Similarly, prolonged exposure to loud noise can cause mechanical and metabolic damage to hair cells, resulting in their apoptosis. Unlike conductive hearing loss, which can often be treated surgically, SNHL typically requires cochlear implants or emerging gene therapies to restore function.

Nanotechnologies have unveiled an expanding landscape of genetic alterations underlying human hearing loss-targets addressable by gene therapy, showing significant promise for treating sensorineural hearing loss (SNHL) in animal models, critical barriers impede clinical translation. These include overcoming the blood-labyrinth barrier, developing targeted delivery systems, refining surgical approaches, and validating novel targets. Furthermore, effective treatment requires overcoming anatomical barriers to achieve localized therapeutic delivery. Recent advances in nanomedicine offer promising solutions: engineered nanoparticles enable sustained, targeted drug release to inner ear structures *via* diffusion through round/oval window membranes. These systems can bypass the blood-labyrinth barrier and focus therapeutics on specific cochlear regions.

Currently, most gene therapies related to hearing loss primarily target SNHL. This is achieved through strategies such as repairing defective genes or restoring function. In contrast, gene therapy approaches are less commonly used for hearing loss related to conductive hearing loss (CHL) or mixed hearing loss. Briefly describe its pathogenesis in the following sections.

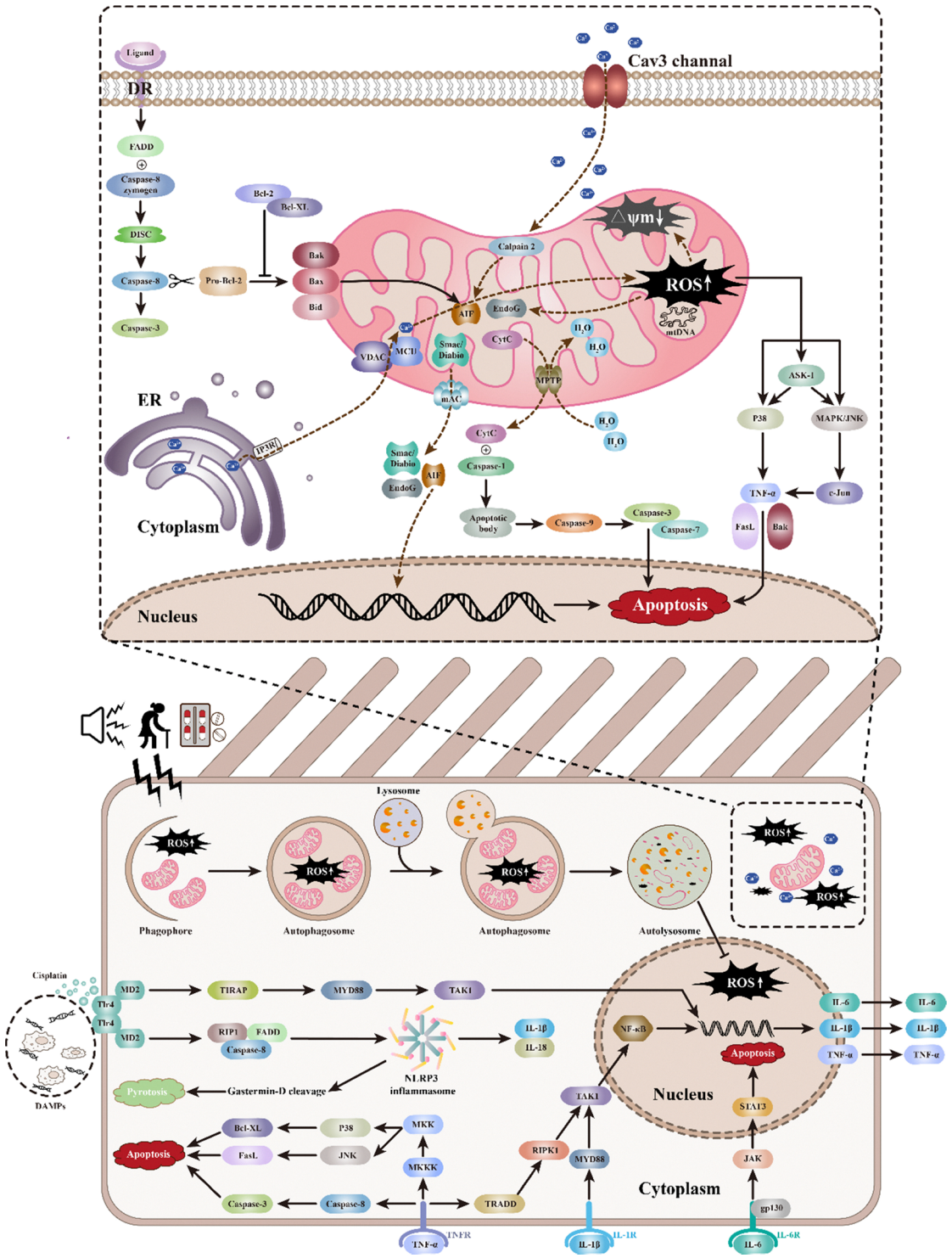

Fig. 1 The pathophysiological interplay among apoptotic pathways, oxidative stress cascades, immune-mediated inflammatory responses, mitochondrial dysfunction, and autophagic regulation impairments in the pathogenesis of sensorineural hearing loss.

1.1.2. Conductive hearing loss (CHL). Conductive hearing loss (CHL) occurs when sound waves cannot efficiently reach the cochlea due to obstructions or abnormalities in the outer or middle ear.[64,65,75,76] This condition differs from SNHL in that it often results from mechanical issues rather than cellular or neural damage. Common causes include earwax blockage, fluid buildup from ear infections (otitis media), otosclerosis (abnormal bone growth in the middle ear), and tympanic membrane perforation.[77,78] These conditions can dampen sound transmission, leading to reduced hearing sensitivity, but they do not affect the auditory nerve or cochlear function directly.

Unlike SNHL, CHL is often temporary or treatable through medical or surgical interventions. Treatments may involve earwax removal, antibiotics for infections, tympanoplasty for eardrum repair, or stapedectomy for otosclerosis. In cases where medical intervention is insufficient, hearing aids or bone-conduction implants can help improve sound transmission. While CHL does not typically result in complete deafness, untreated chronic conditions may lead to complications, including secondary SNHL.

1.1.3. Mixed hearing loss (MHL). Mixed hearing loss (MHL) is a condition in which an individual experiences both sensorineural and conductive hearing impairments simultaneously.[79,80] This means that there is damage to both the cochlea or auditory nerve (inner ear) and the outer or middle ear structures responsible for sound conduction. The severity of MHL varies depending on the degree of impairment in each component, and it can be either congenital or acquired. Individuals with MHL may experience difficulties in sound perception, including reduced clarity, distorted sounds, and challenges in distinguishing speech in noisy environments.

The causes of mixed hearing loss often involve a combination of factors. For example, a person with otosclerosis (a conductive issue due to abnormal bone growth in the middle ear) may also develop age-related cochlear degeneration (sensorineural hearing loss). Similarly, chronic otitis media (middle ear infections) can cause both conductive hearing loss due to fluid buildup and secondary sensorineural damage if the infection spreads to the inner ear. Other contributing factors may include head trauma, which can simultaneously damage both middle ear structures and the auditory nerve, and long-term exposure to loud noise, which may cause mechanical damage to the eardrum while also leading to cochlear hair cell degeneration.

Treatment for mixed hearing loss depends on the underlying causes and severity of each component. Conductive aspects may be addressed through medical or surgical interventions, such as tympanoplasty (eardrum repair), ossiculoplasty (middle ear bone reconstruction), or stapedectomy (surgical treatment for otosclerosis). Meanwhile, the sensorineural component may be managed with hearing aids, cochlear implants, or experimental gene therapy approaches if the damage is severe. In cases where surgical correction is insufficient, bone-conduction hearing devices can bypass the conductive issue and directly stimulate the cochlea.

1.1.4. Advances in gene therapy. Gene therapy represents a groundbreaking approach for restoring hearing at the molecular level, offering potential treatments for both genetic and acquired hearing loss by targeting the root causes of auditory dysfunction.[81] Unlike traditional hearing aids or cochlear implants, which merely amplify or bypass damaged auditory structures, gene therapy aims to correct, replace, or regulate genes responsible for cochlear development, hair cell function, and synaptic transmission. Several strategies have been developed, including gene replacement therapy (Fig. 2), which introduces functional copies of defective genes, and gene silencing therapy, which uses RNA interference (RNAi) to suppress harmful gene expression. More advanced approaches, such as CRISPR/Cas9 genome editing, offer precise correction of genetic mutations, while regenerative gene therapy seeks to stimulate the regeneration of lost hair cells by reprogramming supporting cells within the cochlea.[82] However, clinical translation remains a significant challenge due to gene delivery limitations, safety concerns associated with viral vectors, and the complexity of targeting inner ear structures. Recent advancements in synthetic nanoparticle carriers, engineered adeno-associated viruses (AAVs), and exosome-based delivery systems are helping to overcome these barriers, improving efficiency and safety.[38,83–87] As research continues to evolve, the integration of gene therapy with regenerative medicine, pharmacological interventions, and next-generation biomaterials may provide long-term, personalized treatments for individuals with hearing loss, potentially offering a cure rather than just a temporary solution.

1.2. Technological innovations in nanoparticle gene delivery

In recent years, nanotechnology-based gene delivery systems have made significant strides in the field of inner ear therapy. These innovations encompass advancements in nanoparticle materials, improved targeting strategies, and enhanced controlled-release mechanisms, making gene therapy more precise and efficient. Compared to traditional viral and non-viral delivery methods, nanoparticle-based systems offer higher biocompatibility, lower immunogenicity, and the ability to achieve sustained and localized gene expression, paving the way for safer and more effective treatments for inner ear disorders. Furthermore, the development of novel nanoparticle formulations has enabled the efficient delivery of genetic material across biological barriers, such as the round window membrane (RWM) and blood-labyrinth barrier (BLB), which traditionally pose challenges for inner ear drug delivery. By leveraging surface modifications and ligand conjugation, nanoparticles can be engineered for targeted delivery to specific cochlear or vestibular cells, enhancing therapeutic efficacy while minimizing off-target effects. Additionally, the incorporation of stimuli-responsive materials, such as pH-sensitive or enzyme-degradable polymers, allows for controlled and sustained gene release, ensuring prolonged therapeutic effects. These innovations collectively contribute to overcoming key limitations in conventional gene therapy approaches and hold great promise for treating genetic hearing loss, sensorineural disorders, and age-related hearing impairments with greater precision and safety.

1.2.1. Diverse nanomaterials for gene delivery. Nanoparticles are emerging as effective gene delivery vehicles for inner ear therapies, with lipid-based, polymeric, and inorganic nanomaterials offering unique advantages. Lipid-based nanoparticles (LNPs) are biocompatible and protect genetic cargo from enzymatic degradation.[88–96] They facilitate cellular entry by fusing with membranes and have been successfully used in cochlear gene transfer, including CRISPR–Cas9 delivery for Tmc1 gene editing. Polymeric nanoparticles, including PLGA and PEGylated polyesters, enable controlled cargo release and effective transfection of inner ear cells.[97–102] PEI-PEG nanoparticles have demonstrated high gene delivery efficiency with reduced toxicity, while PLGA nanoparticles have been tested for

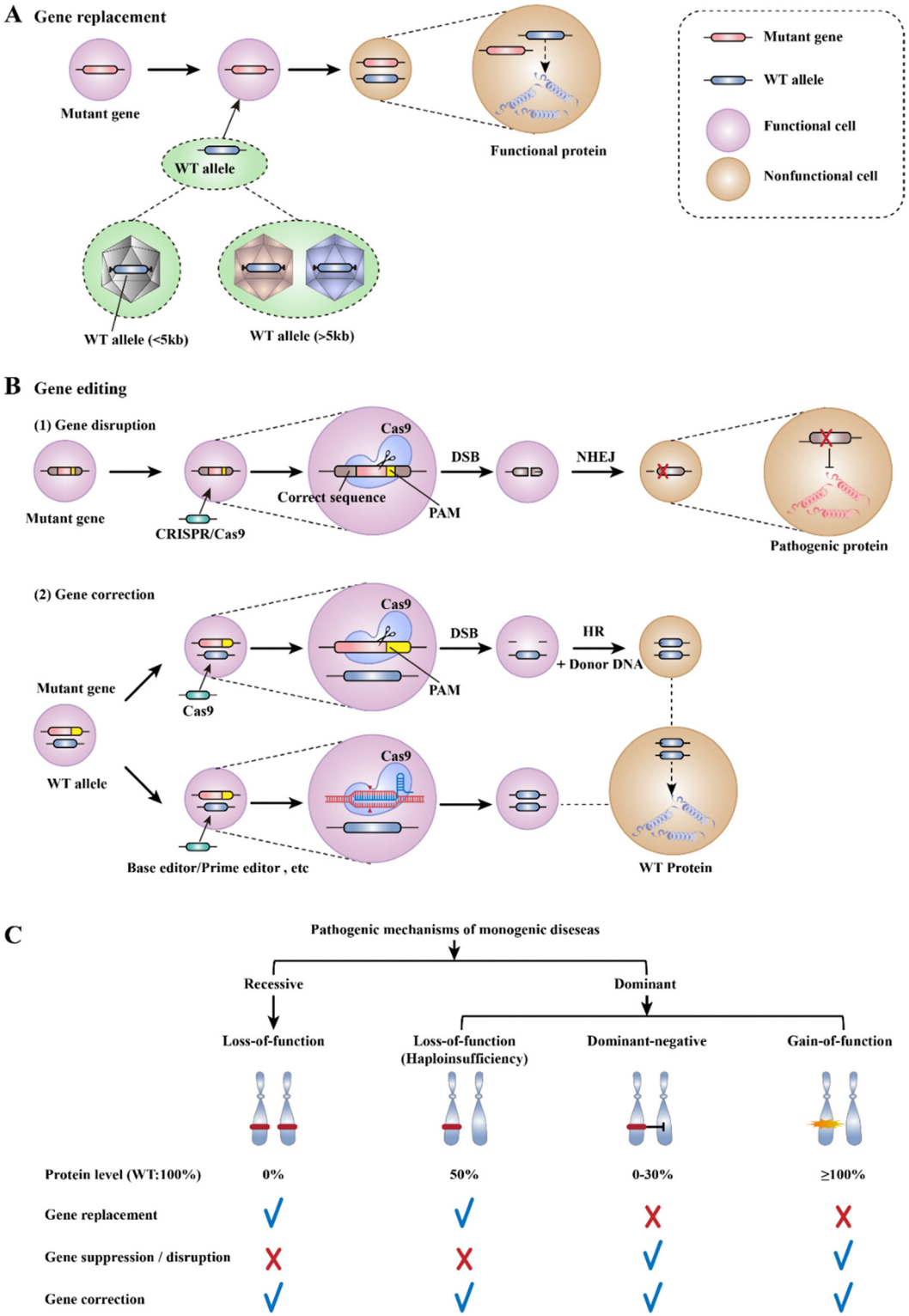

Fig. 2 Gene therapy strategies for monogenic disorders. (A) The major strategies performing gene replacement. (B) The major strategies performing gene editing. (C) Gene-editing therapeutics for monogenic diseases: from pathogenic variants to precision correction. DSB: double-strand breaks, HR: homology-directed repair, NHEJ: nonhomologous end joining, PAM: protospacer adjacent motif.

local drug delivery *via* the round window membrane.[103–105] However, unmodified polycations like PEI can be ototoxic at high doses, necessitating careful biocompatibility design.[106–110] Inorganic nanoparticles, such as gold, silica, and magnetic nanoparticles, offer additional functionalities. Gold nanoparticles can be functionalized with DNA or peptides, while magnetic

nanoparticles (MNPs) can be guided using external magnetic fields to enhance cochlear penetration.[111–118] However, concerns about oxidative stress and toxicity in sensitive ear tissues persist. Recent studies show that PEG-coated MNPs minimize adverse effects, maintaining cochlear cell viability even under magnetic field application.[119,120] Each nanoparticle type presents trade-offs in efficiency, toxicity, and targeting ability, emphasizing the need for optimized designs to maximize gene therapy benefits while ensuring safety for inner ear applications.

1.2.1.1. Lipid-based nanoparticles. Liposomes and lipid nanoparticles (LNPs) are composed of phospholipid bilayers, often with a positive charge to bind DNA/RNA.[121–123] They are biocompatible and low in immunogenicity, making them attractive for inner ear use.[124] Lipid NPs protect genetic cargo from enzymatic breakdown and facilitate entry into cells by fusing with cell membranes.[125–127] A classic example is cationic liposomes, which were the first non-viral vectors used in cochlear gene transfer. LNPs have recently enabled efficient CRISPR–Cas9 delivery for gene editing in the cochlea (as noted with the Tmc1 editing study).[128] This study demonstrates a CRISPR–Cas9-based genome editing approach to treat autosomal dominant hearing loss caused by mutations in the Tmc1 gene (Fig. 3). By using lipid-mediated delivery of Cas9–sgRNA

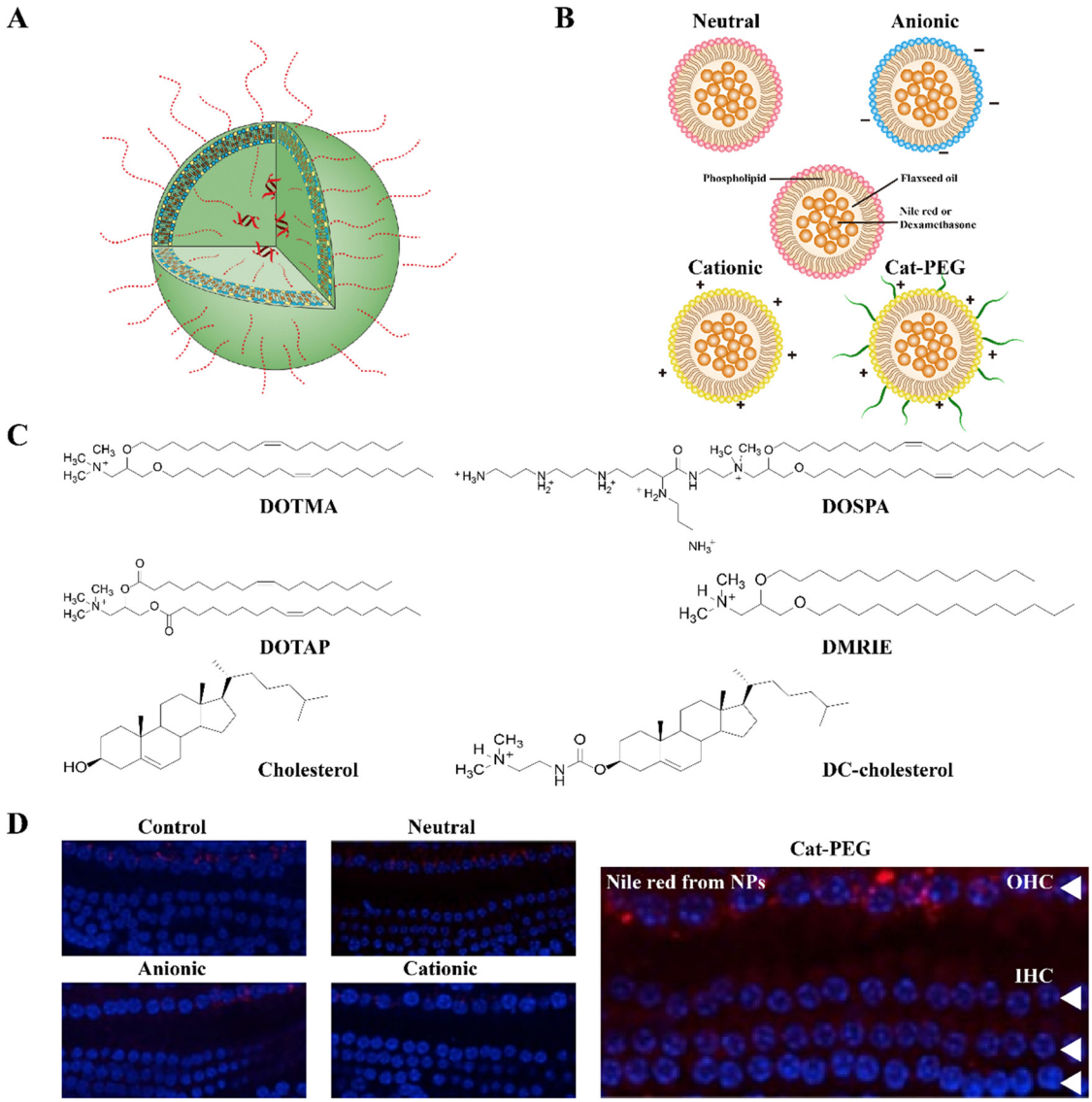

Fig. 3 Structure of non-viral siRNA vector for inner ear drug delivery and therapy. (A) A stable nucleic acidlipid particle (SNALP) SNALP comprises DSPC (helper lipid), cholesterol, cationic lipid (*e.g.*, DLinDMA), and PEG-lipid (PEG-CDMA), forming a bilayer structure. PEG provides a hydrophilic exterior for stability, which later dissociates to expose cationic lipids for cell transfection. Cationic lipids aid cellular uptake, while helper lipids enable endosomal escape.[129] (B) Four phospholipid nanoemulsion-based nanoparticles loaded with Nile red (tracking) or dexamethasone (therapy) are schematically depicted. (C) Cationic lipids (*e.g.*, DOTMA, DOTAP) bind DNA and enhance transfection *via* cationic head groups, hydrophobic tails, and linkers. Neutral lipids (*e.g.*, DSPC, cholesterol) act as helpers to stabilize nanoparticles and improve efficacy. (D) *In vivo*, Cat-PEG nanoparticles showed significantly higher Nile red fluorescence intensity around cochlear hair cells (OHCs/IHCs) compared to other nanoparticles.[130]

complexes, researchers successfully targeted and disrupted the mutant allele in a mouse model (Tmc1Bth/+), leading to higher hair cell survival and improved auditory function.

These lipid-based systems are the same technology underlying mRNA vaccines, repurposed here to carry therapeutic genes to inner ear cells. LNPs share technological foundations with mRNA vaccines, which have been widely deployed for COVID-19 immunization.[102,131–134] Their success in systemic mRNA delivery has spurred renewed interest in adapting these systems for gene therapy applications beyond vaccination. In the context of inner ear treatments.

Further advancements in lipid nanocarrier design are focused on improving targeting specificity and controlled release profiles to enhance therapeutic outcomes.[135] Surface modifications, such as PEGylation or ligand functionalization, are being explored to optimize cochlear uptake while minimizing systemic distribution and potential toxicity. Additionally, researchers are investigating how LNP formulations can be tailored to bypass biological barriers within the inner ear, ensuring precise and sustained gene delivery (Fig. 4).[136] These continuous innovations highlight the transformative potential of lipid-based nanoparticles in advancing gene therapy for hereditary and acquired hearing disorders.

1.2.1.2. Polymeric nanoparticles. Polymers like PLGA (poly-lactic-*co*-glycolic acid), PEGylated polyesters, or cationic polymers (*e.g.* polyethylenimine, PEI) are widely used to form nano-sized gene carriers.[138,139] They are often biodegradable, allowing for slow release of cargo as the polymer matrix breaks down. Polymeric NPs can be engineered to condense large DNA plasmids and release them over time, addressing the size limitation

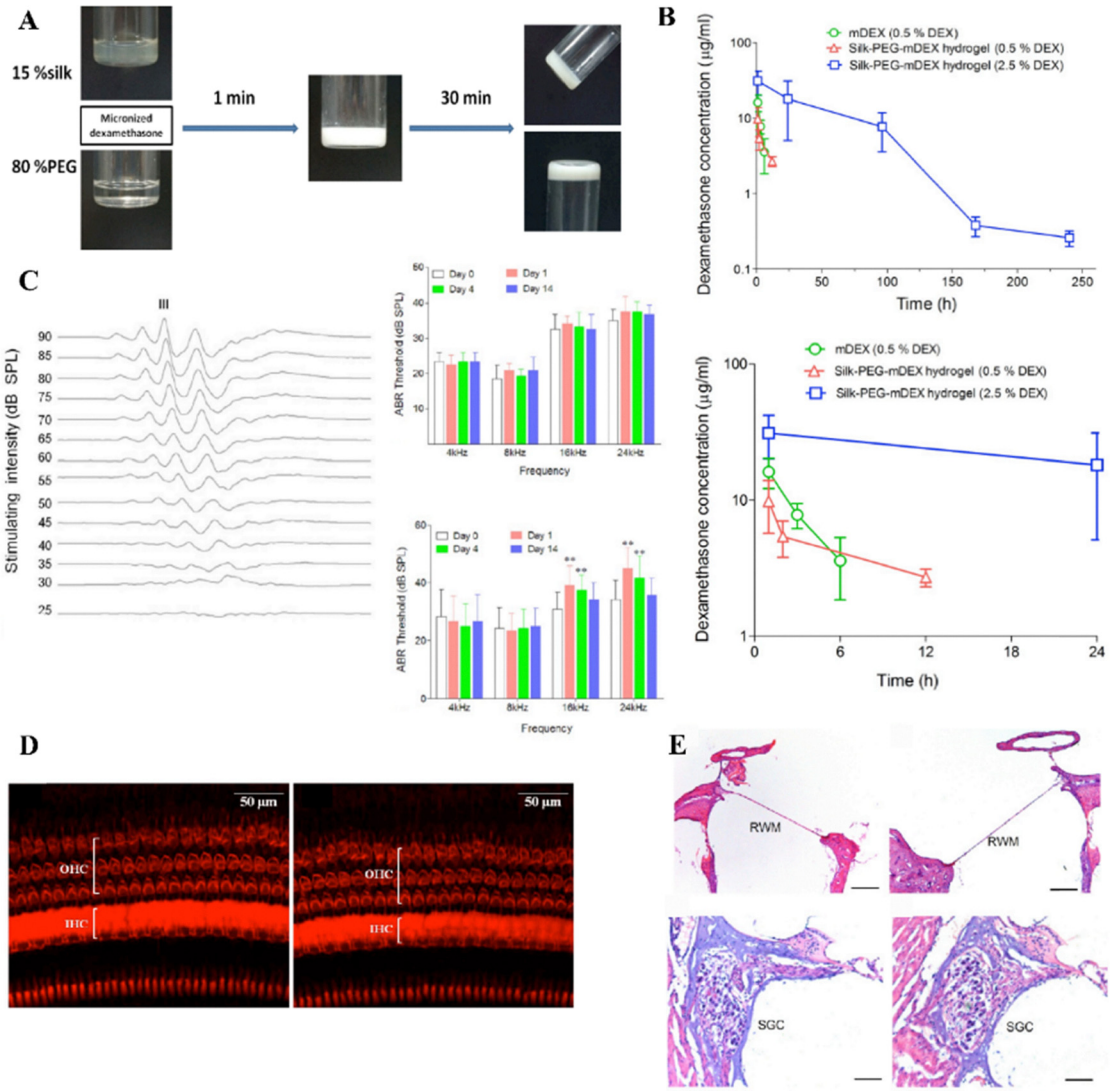

Fig. 4 Silk-PEG-mDEX hydrogel enables sustained cochlear dexamethasone delivery with preserved hearing and tissue safety.[137] (A) Silk-PEG-mDEX hydrogel formed by mixing micronized dexamethasone (mDEX) in silk solution with PEG400, solidifying into a firm 7.5% hydrogel. (B) Higher perilymph DEX concentration after silk-PEG-mDEX hydrogel application *vs.* free mDEX in guinea pigs. (C) Silk-PEG-mDEX hydrogel administration showed no adverse effects on hearing thresholds. (D) Rhodamine phalloidin staining revealed intact basilar membrane structure. (E) Histological sections (H&E staining) sections confirmed no middle/inner ear tissue damage.

of viral vectors.[124] *In vitro* studies have shown polymer-based NPs can transfect inner ear cells effectively; for instance, a PEI-PEG nanoparticle achieved higher gene delivery into spiral ganglion neurons with lower toxicity than a standard liposome agent.[140] PLGA NPs have been tested *in vivo* by placing them on the round window membrane – they were able to permeate into the cochlea and deliver drugs locally. Zhou group utilized Tet1 peptide-functionalized PEG-*b*-PCL polymersomes as a targeted drug delivery system for the cochlear nerve. The researchers aimed to enhance the delivery of therapeutic agents to spiral ganglion cells (SGCs), which are crucial for auditory function. By modifying polymersomes with the Tet1 peptide, which binds specifically to trisialoganglioside (GT1b) receptors on neurons, the study demonstrated improved targeting of cochlear nerve fibers when delivered *via* cochleostomy, but not through transtympanic injection. The results indicate that Tet1-functionalized polymersomes can efficiently reach neural structures, potentially offering a novel strategy for treating sensorineural hearing loss by overcoming limitations in drug penetration and specificity.[141] However, some polycations like unmodified PEI can be ototoxic at higher doses, highlighting the need for careful design to ensure biocompatibility[142] (Fig. 5).

1.2.1.3. Inorganic nanoparticles. Inorganic nanomaterials (such as gold nanoparticles, silica nanoparticles, or magnetic nanoparticles made of iron oxide) are also being explored for cochlear gene therapy.[144,145] These offer unique functionalities: gold NPs can be easily functionalized with DNA or peptides, and magnetic NPs (MNPs) can be guided or retained in the inner ear using external magnetic fields. Magnetic nanocarriers are promising for enhancing drug/gene penetration into the cochlea, but they raise concerns about potential oxidative stress or toxicity in sensitive inner ear tissues.[146] This study utilized magnetic nanoparticles (MNPs) with PEG coatings for cochlear drug delivery. The researchers synthesized iron oxide-based MNPs with precisely controlled coating thickness and size, using PEG-12 and PEG-3000 polymers to enhance biocompatibility and optimize drug transport. Results demonstrated that both outer and inner hair cells, as well as spiral ganglion neurites, remained intact after exposure to MNPs, even under a magnetic field. This research addresses the challenge of safe and effective drug delivery to the cochlea, offering a customizable, non-invasive platform for inner ear therapies (Fig. 6). Recent work has focused on synthesizing magnetic NPs with coatings (*e.g.* PEG) to improve biocompatibility; in mouse organ-of-Corti cultures, such optimized MNPs showed no significant hair cell or neuron loss even when a magnetic field was applied to concentrate them. Inorganic NPs can also serve as imaging agents (for example, superparamagnetic iron oxide provides MRI contrast to track distribution in the ear).[147,148] While not as extensively studied as lipid or polymer vectors in the ear, inorganic NPs expand the toolkit for delivery and monitoring of gene therapies.

1.2.1.4. Advantage and disadvantage of non-viral vectors. Non-viral vectors for gene delivery, including cationic lipids, polymeric nanoparticles, and electroporation, offer promising alternatives to viral vectors for inner ear gene therapy. Cationic lipids are advantageous due to their low immunogenicity, well-controlled production, and the ability to carry larger transgenes, but their efficiency in gene transfer is generally lower than that of viral vectors. Polymeric nanoparticles, such as PLGA, provide good efficiency and can cross the round window membrane, allowing for targeted gene delivery to the inner ear. However, they may induce side effects, including hearing loss. Electroporation offers high efficiency by using electrical pulses to deliver genetic material directly into cells, but it is primarily limited to surface cells, and its potential for tissue damage remains a concern. While these non-viral methods are less immunogenic and offer more flexibility in terms of transgene size, they face challenges in efficiency, stability, and long-term effects. Nonetheless, with ongoing improvements in nanoparticle design and delivery methods, non-viral vectors hold significant promise for clinical applications in treating genetic hearing loss.

1.2.2. Targeting strategies and surface functionalization. A major innovation in nanomedicine is the ability to functionalize the NP surface for targeted delivery. PEGylation (adding polyethylene glycol chains) is commonly used to increase nanoparticle stability and stealth, reducing clearance and aggregation. On PEGylated NPs, only a small percentage of surface (1–2%) needs to be occupied by functional ligands to achieve targeting.[150] Targeting ligands can be peptides or antibodies that recognize cell-specific markers in the cochlea. For example, the TrkB peptide mentioned earlier is one such ligand to direct NPs to neurons.[151] Likewise, cell-penetrating peptides like HIV TAT can be attached to help the nanoparticle cross cell membranes and even nuclear membranes, boosting transfection efficiency.[141] These modular surface modifications allow a ''mix-and-match'' design: one can attach an inner ear tissue-targeting moiety, an endosomal escape agent, and an imaging tag all onto the same nanoparticle. This multi-functional approach is exemplified by an ''ideal'' cochlear gene therapy nanoparticle design proposed in the literature. It envisions a NP carrying a combination payload (DNA, siRNA, and drugs) and decorated with various functional elements for maximum efficacy.[152] The schematic shows a liposome-based carrier with a PEGylated surface (white spheres with teal coating) for stability and stealth. It is functionalized with targeting ligands (blue Y-shaped structures, *e.g.* TrkB-peptide) and transfection-enhancing peptides (green tags, *e.g.* TAT) on the surface. Inside the nanoparticle core are therapeutic payloads (red circles) such as genes, siRNA, and drugs, as well as imaging/contrast agents (yellow star shapes, *e.g.* SPION for MRI visibility). This design integrates targeting, delivery, and diagnostic functions into a single nanocarrier (Fig. 7).

1.2.3. Controlled release mechanisms. Unlike one-time bolus delivery with viral vectors, nanoparticle systems allow controlled and sustained release of therapeutic agents in the cochlea. For instance, a drug or DNA encapsulated in a PLGA nanoparticle will be released gradually as the polymer degrades, providing a prolonged therapeutic effect in inner ear tissues. This is beneficial

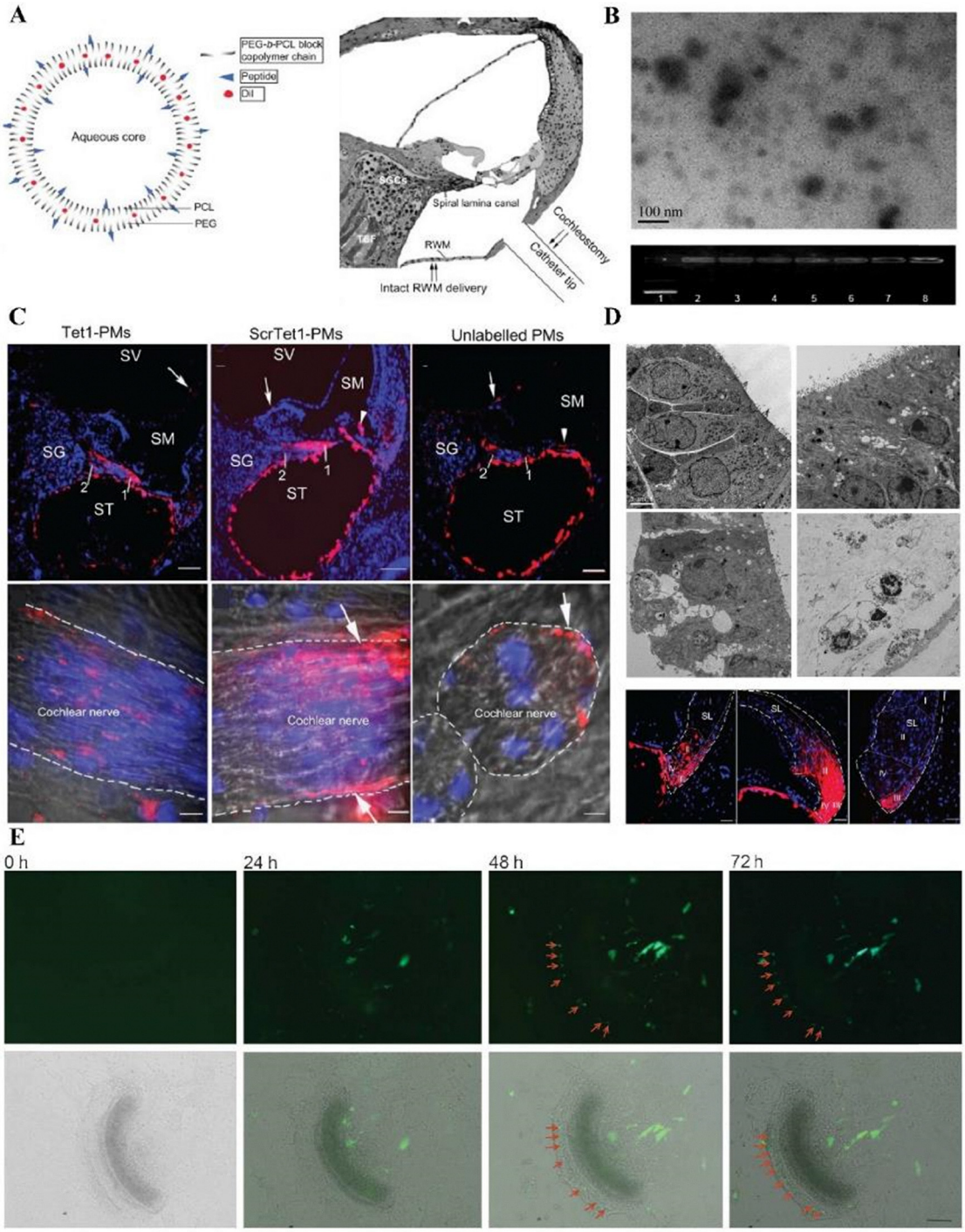

Fig. 5 Tet1-functionalized PMs enable targeted cochlear delivery with sustained transfection and reduced toxicity. (A) Polymersomes (PMs) delivery routes: topical RWM, transtympanic injection, and cochleostomy. (B) LPEI/pDNA polyplexes formed *via* electrostatic interaction, confirmed by TEM and electrophoresis. (C) Tet1-PMs, ScrTet1-PMs, and unlabeled PMs localized in cochlear mesothelial cells (ST/SV) and spiral ganglion. (D) TEM revealed L-PEI/plasmid toxicity and Tet1-PMs' enhanced spiral ligament distribution *vs.* non-functionalized PMs.[143] (E) Sustained eGFP expression (>72 h) in sensory epithelium supporting cells post-transfection.[107]

for chronic conditions or when the therapy needs to act over an extended period (*e.g.* supporting cell reprogramming which may require persistent gene expression). Studies have shown that drugs packaged in nanoparticles and placed on the round window membrane achieve measurable cochlear concentrations without quick clearance.[154,155] In one experiment, PLGA NPs loaded with a corticosteroid (dexamethasone) and applied to the round window provided sustained drug delivery into the cochlea, potentially reducing trauma-related inflammation over time.[156] Similarly, researchers delivered lidocaine *via* nanoparticles on the round

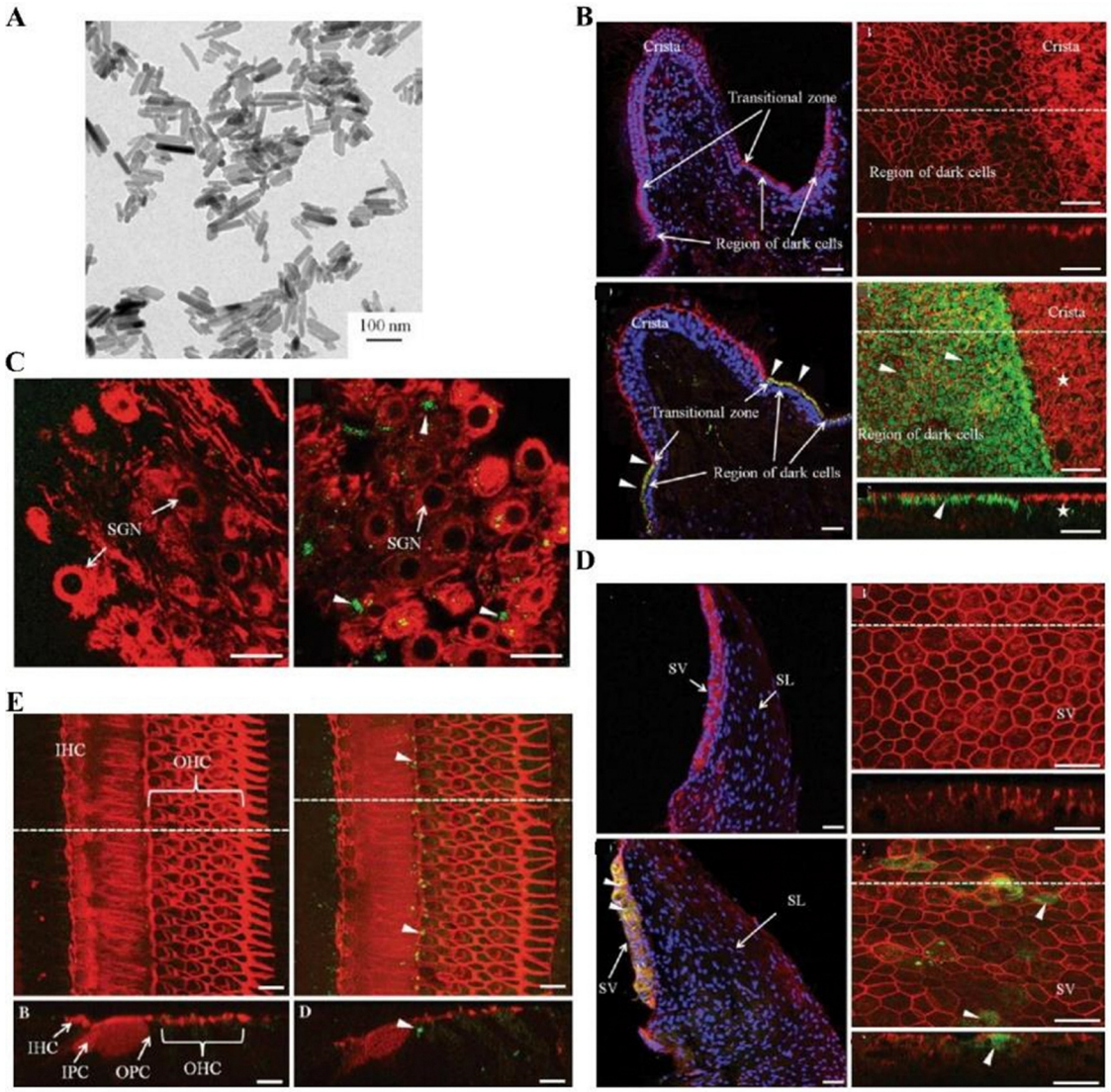

Fig. 6 PEInHAT enables targeted gene expression in cochlear regions, sparing hair cells. (A) PEInHAT nanoparticles (50–100 nm) were synthesized. (B) EGFP expression localized to crista ampullaris transitional/dark cells (not hair cells) post-PEInHAT transfection. (C) EGFP signals detected in spiral ganglion Schwann cells (not neurons). (D) Stria vascularis showed specific EGFP-positive cells post-transfection. (E) Basilar membrane EGFP expression restricted to outer pillar cells (not hair cells).[149]

window and observed effective inner ear drug levels with minimal systemic absorption. Nanoparticles can also be embedded in hydrogels or matrices that stick to the round window niche, acting as a slow-release reservoir. An example is the use of a gelatin hydrogel with insulin-like growth factor 1 (IGF-1) in combination with nanoparticles, which has been tested to treat sudden hearing loss[157] (Fig. 8). This localized delivery approach showed hearing improvement and has progressed to early clinical trials.

Triggerable release is another innovation: some NPs are designed to release their cargo in response to triggers like pH changes (exploiting the acidic environment of endosomes) or external stimuli. As noted, endosome-disruptive peptides can be masked with pH-sensitive bonds that break in acidic conditions, unleashing the peptide's activity exactly when the nanoparticle is taken up by the cell.[160] This kind of smart release greatly enhances the efficiency of gene transfer, as more DNA/RNA escapes into the cytoplasm rather than being trapped in lysosomes.

1.2.4. Advantages over traditional delivery systems. Nanotechnology-based gene delivery offers several advantages over traditional viral vectors and conventional methods. Unlike viral vectors such as AAVs, which have a <5 kb cargo limit, nanoparticles can carry large or multiple genes, enabling treatments for conditions that viral systems cannot address.[161] Peter Colosi reported that both AAV2 and AAV5 exhibit a strict packaging limit of ~ 5.2 kb for intact, full-length genomes. Larger vectors (>5.2 kb) fail to package completely, resulting in truncated, heterogeneous genomes capped at ~ 5 kb due to mechanistic size constraints (Fig. 9A).[162] While AAV capsids impose a strict

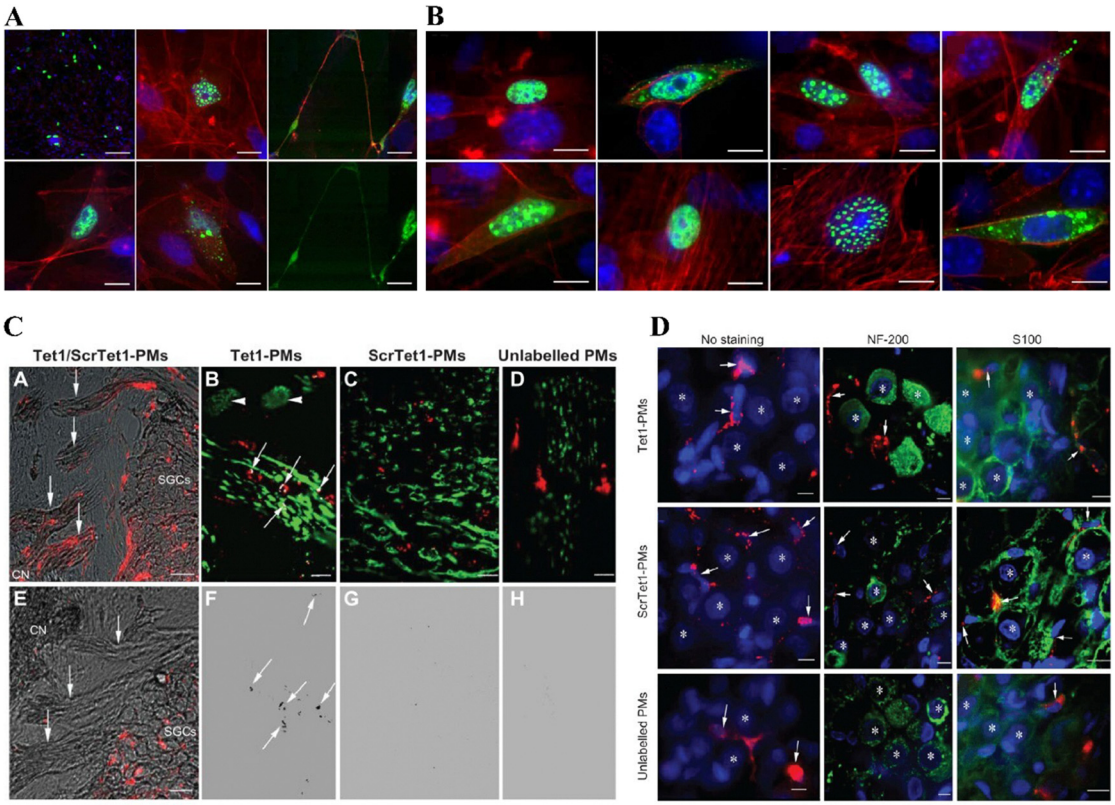

Fig. 7 EmGFP-Math1 exhibits dual localization/vesicles and Tet1-PMs enable nerve-targeted cochlear delivery. (A) Lipofectamine 2000-transfected cochlear cells showed EmGFP-Math1 nuclear/cytoplasmic localization (2.9% efficiency) and vesicle formation (0.4–2.3 μm). (B) NIH 3T3/MSCs exhibited EmGFP-Math1 nuclear dominance (*vs.* cytoplasmic BDNF control), with partial dual localization and vesicles.[153] (C) Tet1-PMs uniquely localized to cochlear nerve fibers (NF-200 co-staining) post-cochleostomy, unlike ScrTet1/unlabeled PMs. (D) PMs accumulated in spiral ganglion satellite cells (not neurons) regardless of Tet1 functionalization.[143]

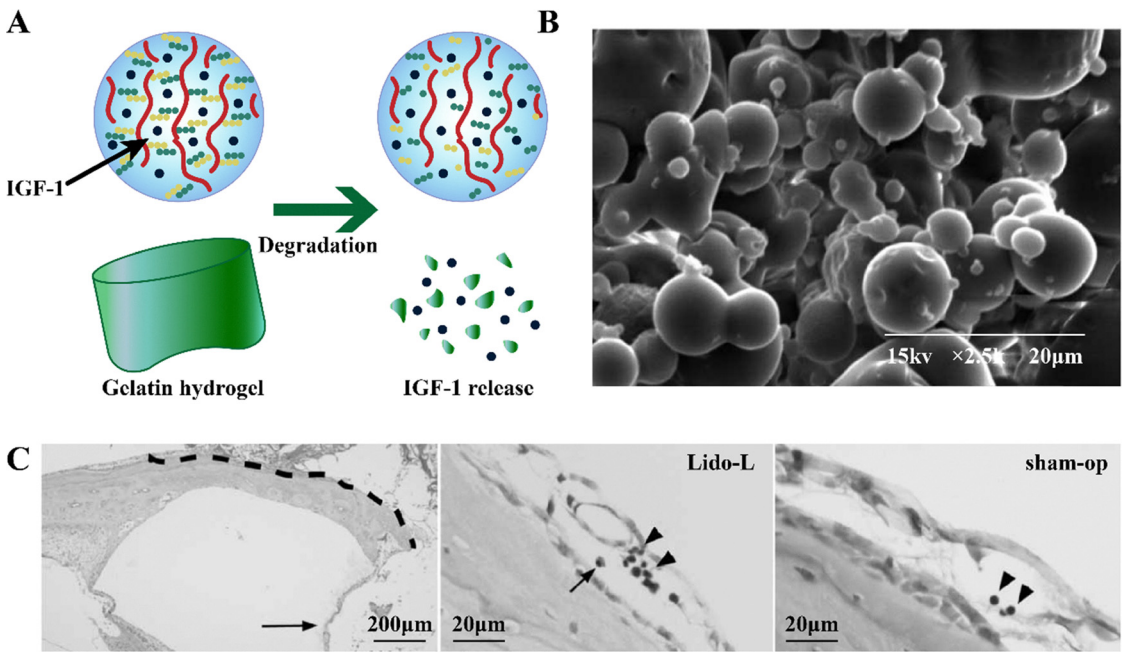

Fig. 8 Gelatin/PLGA-based DDS enables controlled release with localized biocompatibility. (A) Gelatin hydrogel DDS gradually releases IGF-1 *via* polymer degradation. (B) PLGA microparticles encapsulate lidocaine, visualized by SEM.[158] (C) Lido-L microparticles caused mild lymphocytic/neutrophilic infiltration in middle ear mucosa *vs.* sham controls.[159]

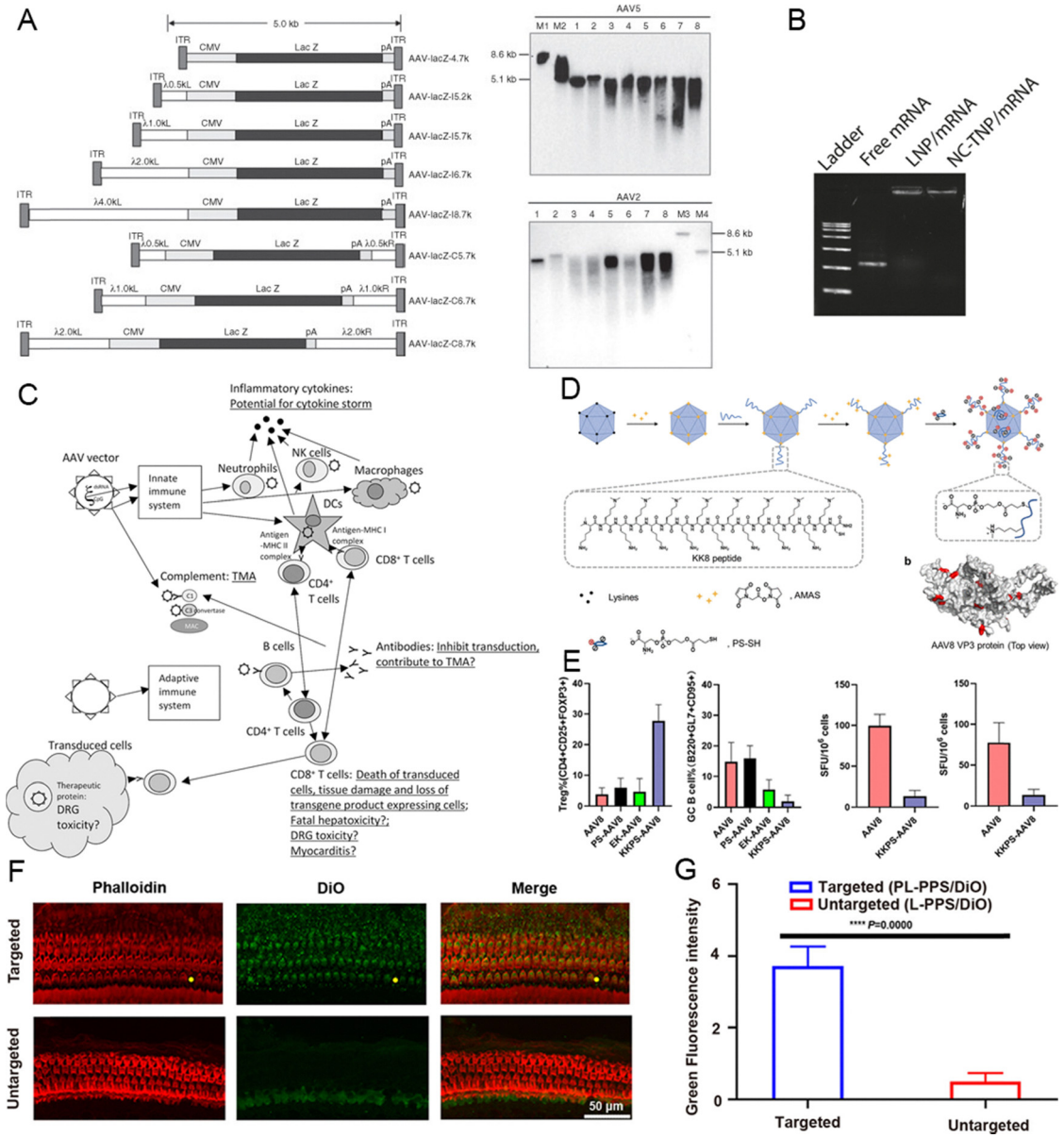

Fig. 9 The advantage of nanotechnology methods. (A) The optimal payload size for AAV vectors is approximately 4.5 kb (as validated by the 4.7 kb vector with a 4.4 kb expression cassette + minimal ITRs). Beyond this range, packaging efficiency declines significantly, governed collectively by transgene position (ITR-proximal > central) and serotype (AAV5 > AAV2).[162] (B) Gel electrophoresis of free mRNA, nanoparticles encapsulated mRNA platform to show the mRNA condensation condition.[90] (C) The graph illustrates immune responses triggered by AAV vector administration.[163] (D) Illustration of the conjugation strategy for preparing zwitterionic phosphoserine-containing peptide-conjugated AAV vectors. (E) Immune profiling: frequency of regulatory T cells (Foxp3$^+$) in CD4$^+$CD25$^+$ splenocytes. Activated germinal center B cell frequency (CD19$^+$GL7$^+$CD95$^+$). AAV8-specific IFN-γ secretion by splenocytes (ELISPOT). Anti-AAV8 IgG-secreting cells (ELISPOT).[164] (F) Representative confocal images demonstrating preferential accumulation of Prestin-targeted nanoparticles (PL-PPS/DiO, green) *vs.* non-targeted controls (L-PPS/DiO) in phalloidin-stained OHCs (red). (G) Quantification of intracellular DiO fluorescence intensity within OHCs (****$p < 0.0001$; $n = 5$). Scale bar: 50 μm.

size limitation of ~5.2 kb for packaging intact genomes (as demonstrated in our studies), multiple research teams—including ours—have successfully delivered larger genes (>4.5 kb) using engineered nanoparticle delivery systems, thereby overcoming AAV's physical constraints (Fig. 9B).[90] Additionally, nanoparticles exhibit lower immunogenicity, reducing the risk of immune responses or neutralizing antibodies, which is especially beneficial for repeat dosing and pediatric treatments. AAV vectors trigger innate immune responses through pathogen-associated motifs (*e.g.*, unmethylated CpG DNA, dsRNA) sensed by dendritic cells (DCs), leading to complement activation (C1/C3 → MAC formation) and natural killer (NK) cell recruitment. These responses initiate adaptive immunity, including MHC-mediated antigen presentation, cytotoxic T-cell clearance of transduced cells, and neutralizing antibody production. Key toxicities linked to these mechanisms include: (1) hepatotoxicity (driven by T-cell

infiltration/complement); (2) thrombotic microangiopathy (TMA) (mediated by complement-induced endothelial damage); and (3) dorsal root ganglia (DRG) injury (potentially involving NK cells/complement, though this remains to be fully elucidated). Critical knowledge gaps persist regarding the roles of dsRNA, NK cells in neurotoxicity, and predictive biomarkers for TMA (Fig. 9C).[163] Another key advantage is targeting specificity—nanoparticles can be engineered to deliver genetic material to specific cochlear cells while avoiding non-target cells, minimizing damage to delicate auditory structures.[164,165] To overcome immune barriers limiting AAV re-administration, Jiang group modified viral vectors functionalized with non-viral delivery systems to reduce and mitigate immunogenicity.[164] He engineered AAVs cloaked in an immunosuppressive zwitterionic phosphoserine polypeptide that mimics apoptotic cell tolerance. These modified vectors evade neutralizing antibody responses while retaining transduction efficacy, enabling successful repeat dosing and functional gene correction in hemophilia A mice (Fig. 9D and E). Liu group also prepared dual-functional nanoparticles (PL-PPS/BBR) engineered with Prestin-targeting peptides and ROS-sensitive polymers to berberine to outer hair cells, effectively reducing oxidative stress/inflammation, protecting hair cell integrity, and reversing hearing loss in preclinical NIHL models, demonstrating strong clinical potential.[165] Prestin-targeting peptide 2-modified nanoparticles effectively targeted OHC areas (Fig. 9F and G). In terms of manufacturing and safety, nanoparticles are chemically synthesized, making them easier and more cost-effective to produce at scale while eliminating risks such as insertional mutagenesis associated with viral vectors. Furthermore, nanoparticles enable multifunctional therapies, allowing simultaneous delivery of genes, siRNA, and drugs, which is particularly valuable for treating complex auditory disorders. The main challenge is lower transfection efficiency compared to optimized viral vectors, but ongoing advancements in surface chemistry, payload design, and delivery methods are rapidly improving their efficacy. With continued innovation, nanotechnology holds great promise for safer and more versatile gene therapies for hearing loss.

1.3. Biological mechanisms of nanotech-assisted gene therapy

NPs offer a promising strategy for gene delivery and editing in the inner ear, protecting DNA, RNA, or CRISPR components from degradation while enhancing cellular uptake. Functionalized NPs can target specific cochlear cells using ligands like TrkB peptides, improving precision and reducing off-target effects. Lipid NPs have successfully delivered CRISPR–Cas9 to edit the Tmc1 gene, preventing hereditary hearing loss in mice. Additionally, NPs enable hair cell regeneration by delivering genes like Atoh1, which induces supporting cells to transdifferentiate into hair cells. They can also transport growth factor genes (*e.g.*, BDNF) to promote neuronal survival or siRNA to silence genes that inhibit regeneration. These advances highlight how nanotechnology can correct genetic defects and activate regenerative pathways, offering a non-viral, efficient approach to restoring hearing.

1.3.1. Nanoparticle-facilitated gene delivery. NPs serve as tiny carriers that can ferry therapeutic DNA, RNA, or even gene-editing proteins into cochlear cells. By complexing with or encapsulating genetic material, NPs shield it from degradation by enzymes and immune neutralization, allowing more DNA or RNA to reach target cells.[127] For example, cationic lipid NPs form protective complexes with nucleic acids, promoting cellular uptake *via* endocytosis. Once inside the cell, engineered features such as endosomal escape peptides (*e.g.* pH-sensitive or TAT peptides) help release the genetic payload into the cytoplasm and eventually the nucleus. This efficient intracellular delivery is crucial for gene therapies to exert their effects.

1.3.2. Cochlear cell targeting. Nanotechnology enables cell-specific targeting in the inner ear. NPs can be functionalized with ligands or antibodies that bind to receptors unique to certain cochlear cells (hair cells, supporting cells, or neurons). An illustrative case is the use of a brain-derived neurotrophic factor (BDNF) mimicking peptide (TrkB ligand) on liposome NPs to target TrkB receptors in cochlear neurons. In a study, liposomes coated with a TrkB affinity peptide delivered a gene-silencing shRNA into cochlear cells; these targeted NPs showed greater gene expression of the therapeutic payload compared to non-targeted NPs (Fig. 10). Importantly, both targeted and untargeted NPs were internalized by inner ear cells (*e.g.* spiral ganglion neurons) *in vivo*, but the ligand-enhanced NPs achieved higher therapeutic efficacy. This demonstrates that nano-carriers can be ''aimed'' at specific cell types, improving precision and reducing off-target effects.

1.3.3. Gene editing in the inner ear. Nanoparticles also facilitate advanced gene editing (*e.g.* CRISPR–Cas9) for hearing loss. Rather than relying on viral vectors, researchers have used lipid NPs to deliver CRISPR components directly to cochlear cells. In one landmark study, cationic lipid NPs were loaded with Cas9 protein and a guide RNA targeting the Tmc1 gene (a mutation in Tmc1 causes hereditary deafness) (Fig. 11). After injection into the cochlea (scala media) of Beethoven mutant mice, the NP-delivered CRISPR system selectively disrupted the defective Tmc1 allele.[128] Treated mice showed enhanced hair cell survival and reduced hearing loss progression compared to controls. This example highlights how nanotech can enable allele-specific gene editing *in situ*, potentially correcting dominant-negative mutations that cause deafness. Notably, the non-viral CRISPR approach avoided issues of viral vectors (like size limits for Cas9 or immune reactions) and succeeded in ameliorating hearing in an animal model of genetic deafness.

Current therapeutic advances with emphasis on genetic-based nanotechnology as a promising research avenue. Nanotechnology combined with novel excipients represents a strategic focus for future hearing restoration research, though further investigation is warranted. Based on the distinct types of inner ear cells, nanodelivery systems can be designed with specific targeting capabilities, enabling precise delivery to treat hearing-related disorders—representing a promising therapeutic approach (Fig. 12A). A biomarker-a biological molecule indicating disease status—can identify site-specific damage in acquired cochlear pathologies. Each inner cell type expresses unique

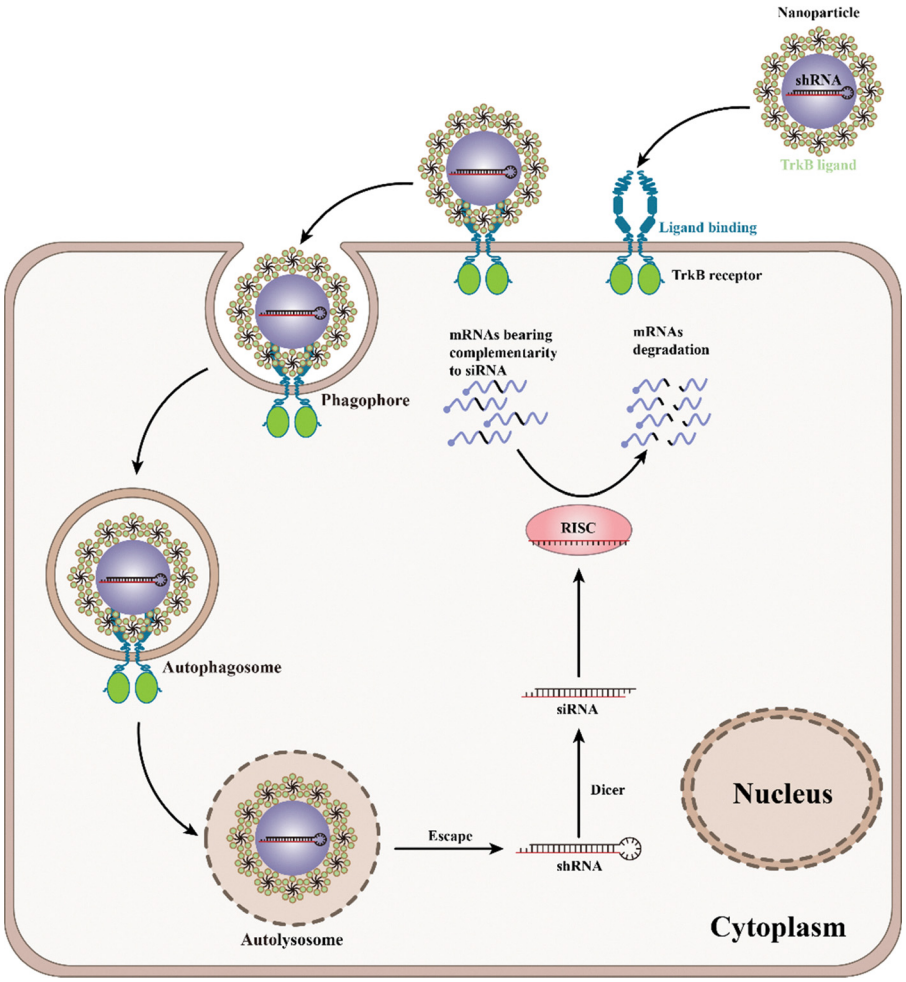

Fig. 10 Schematic illustration of liposomes coated with a TrkB affinity peptide delivered a gene-silencing shRNA into cochlear cells.

molecular biomarkers (typically proteins) (Fig. 12B).[166] Critically, acquired injuries often target specific sites: cisplatin damages outer hair cells,[167] noise toxicity affects spiral ganglion cells in synaptopathy, and loop diuretics target the stria vascularis. This specificity enables targeted therapies for cochlear injuries. However, effective drug delivery requires biomarkers with accessible extracellular domains for precise interaction. Researchers have engineered peptide-conjugated nanodelivery systems that specifically target validated surface markers unique to distinct cell types, thereby achieving cell-type-specific delivery within the inner ear. Cell-specific targeting represents an emerging frontier in nanomedicine, achieved through surface functionalization of multifunctional nanoparticles (MFNPs) with targeting moieties. The inner ear presents a compelling therapeutic target for novel delivery strategies due to its anatomical inaccessibility and the global burden of hearing loss. Schrott-Fischer group prepared nerve growth factor-derived peptide (hNgf EE)-functionalized nanoparticles for targeted delivery to inner ear cells (Fig. 12C).[168] These NPs in mouse organotypic inner ear explants and PC-12 rat pheochromocytoma cells, observing no toxicity. Ligand-mediated multivalent binding to tyrosine kinase receptors and p75 neurotrophin receptors enabled specific targeting with enhanced binding affinity to spiral ganglion neurons, Schwann cells, and nerve fibers in explant cultures to the inner ear (Fig. 12D). Zou group prepared the polymersome modified with Tet 1peptide sequence to target to specific cells witnin the inner ear (Fig. 12E).[143]

1.3.4. Regeneration of cochlear cells. Beyond correcting genes, nanotechnology-assisted gene therapy can drive regenerative processes in the inner ear.[169,170] One strategy is delivering genes that convert supporting cells into new hair cells. For instance, the Atoh1 (Math1) gene is a master regulator that can induce supporting cells to trans-differentiate into hair cell-like cells. Researchers have used liposomal NPs to deliver an Atoh1 expression plasmid to inner ear cell cultures, achieving expression of the gene in cochlear cells.[171,172] The efficiency of gene transfer depended on NP design (size, surface charge, PEGylation), and successful nuclear trafficking of the Atoh1 protein was observed, indicating that the delivered gene reached its target inside the cells. Such induced hair cell regeneration could restore hearing by replacing lost sensory cells. In parallel, NPs can deliver growth factor genes or proteins to promote cochlear repair.[173] As an example, early studies transplanted cells secreting BDNF (a neuron-supportive factor) into the

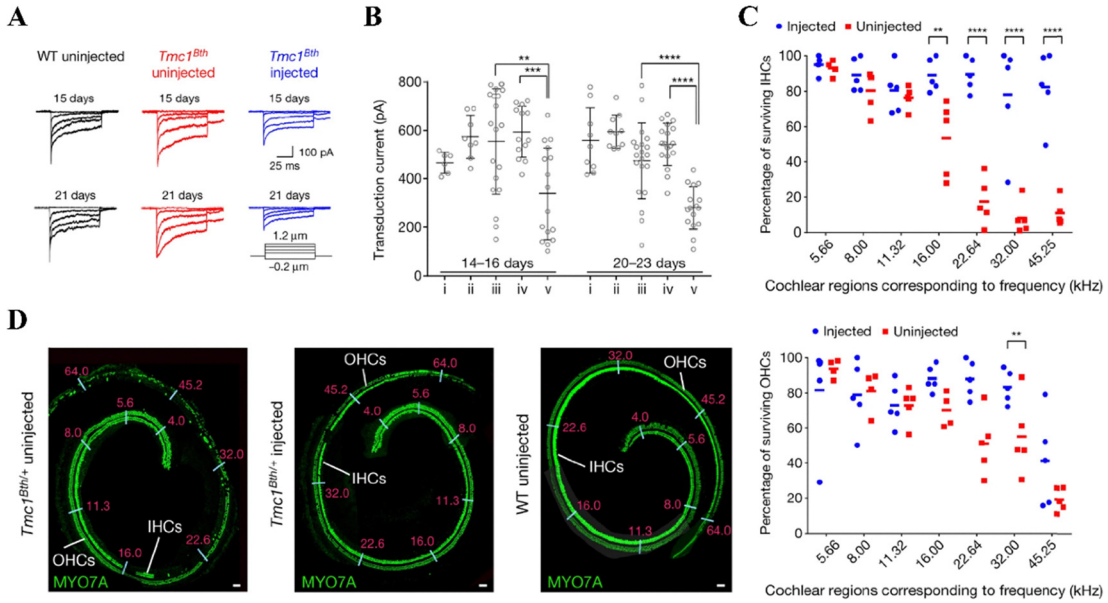

Fig. 11 Cas9-Tmc1-mut3-lipid rescues hair cell function/survival in Tmc1-mutant mice. (A) Cas9-Tmc1-mut3-lipid restored IHC transduction currents in Tmc1Bth/ΔTmc2Δ/Δ mice by day 15–21 post-injection. (B) Tmc1-mutant mice exhibited significantly improved maximal transduction currents after Cas9-Tmc1-mut3 treatment *vs.* controls. (C) Cas9-Tmc1-mut3 increased IHC/OHC survival to near-wildtype levels (100%) at 8 weeks post-treatment. (D) Confocal imaging confirmed cochlear preservation in treated Tmc1Bth/+ mice, resembling wildtype morphology.[25]

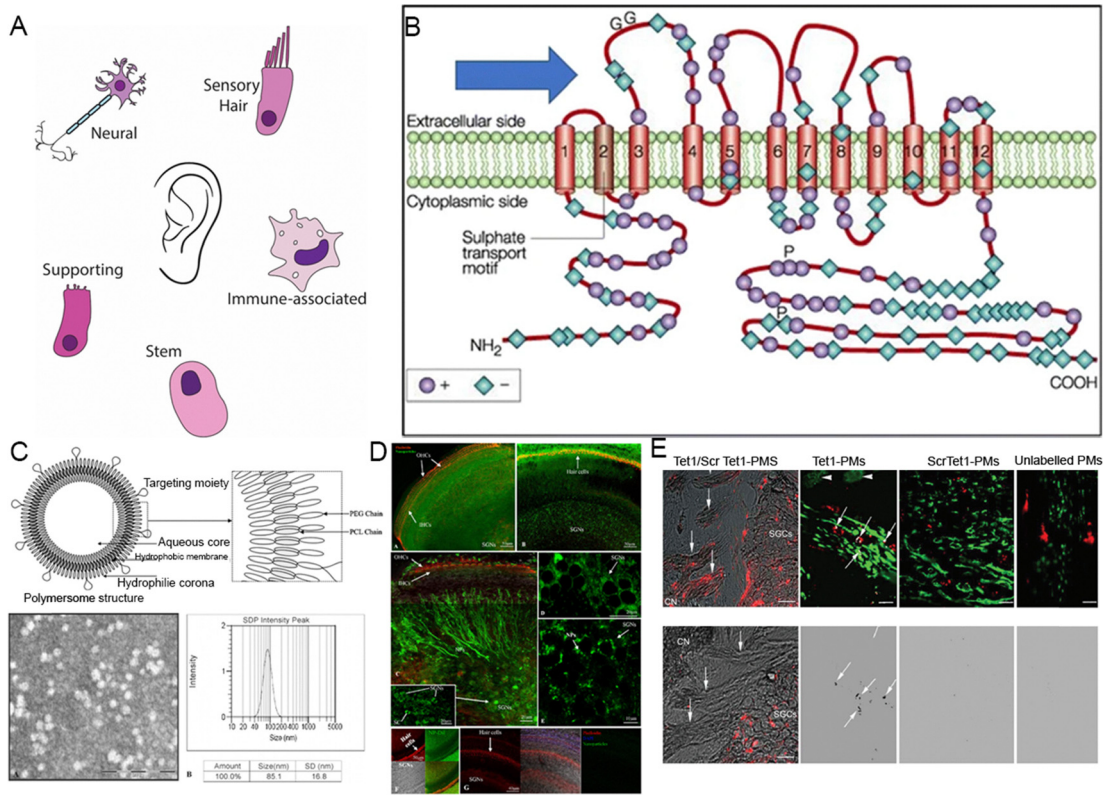

Fig. 12 Inner ear cells targeting. (A) The types of inner ear cells. (B) Prestin exemplifies a targeted drug delivery biomarker.[166] (C) PEG-*b*-PCL copolymers spontaneously form targeted drug carriers (polymersomes) in water. Their design leverages hydrophobic PCL membrane cores and dual-positioned PEG: corona-facing for biocompatibility and lumen-facing for hydrophilic cargo encapsulation. Targeting moieties are conjugated to the nanoparticle surface for cell-specific delivery.[168] (D) The cell targeting ability of nanoparticles. (E) Distribution of the polymersome modified with Tet 1peptide sequence within the cochlear nerve following cochleostomy administration.[143]

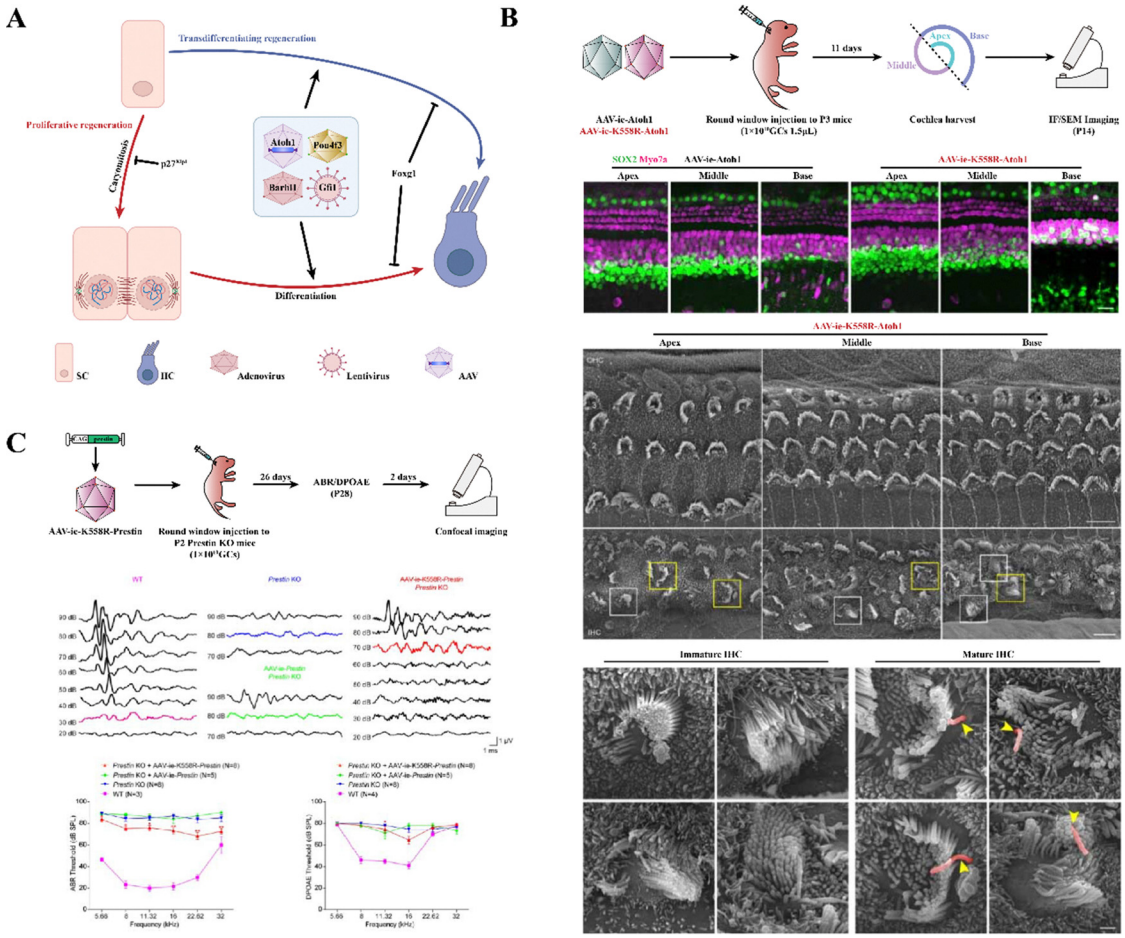

Fig. 13 AAV-ie-K558R safely delivers therapeutic genes to restore hearing *via* HC regeneration. (A) Gene therapy enables hair cell (HC) regeneration *via* SC transdifferentiation or proliferation by delivering HC-related genes to cochlear non-sensory cells. (B) AAV-ie-K558R demonstrates safety with no HC loss (SEM), preserved hearing (ABR/DPOAE), and no structural damage in injected cochleae *vs.* controls. (C) AAV-ie-K558R-Prestin rescues auditory function in Prestin KO mice, showing significantly improved ABR/DPOAE thresholds compared to untreated controls.[169]

cochlea using liposome transfection, which led to increased BDNF levels and improved neuronal survival[174] (Fig. 13). Today's NP systems could similarly carry genes encoding neurotrophic factors to encourage regeneration of auditory neurons or protect them from damage. Additionally, gene silencing *via* siRNA is a mechanism to preserve hearing in certain cases – NPs can deliver siRNA to knock down genes that impede hair cell recovery. In a proof-of-concept, silencing the Id2 gene (which inhibits hair cell differentiation) with shRNA-loaded NPs enhanced the potential for supporting cells to become hair cells in adult rat cochleae.[175] Taken together, these examples show that at the molecular level nanocarriers can introduce DNA or RNA therapies into cochlear cells to either correct genetic defects or activate regenerative pathways, offering hope for true biological restoration of hearing function.

1.4. Clinical applications and challenges

Nanoparticle-based gene therapy is emerging as a promising alternative to viral vectors for treating hearing loss. Unlike AAV-based therapies, which are limited by gene size constraints and immune responses, NPs can deliver large or multiple genetic payloads, including CRISPR, siRNA, and transcription factors. Studies have demonstrated that shRNA-loaded NPs silencing the Id2 gene enhanced supporting cell differentiation into hair cells in adult rat cochleae, offering a potential regenerative treatment. Similarly, CRISPR-loaded lipid NPs successfully edited the Tmc1 gene in mice, preventing hereditary deafness. Additionally, NPs carrying Atoh1 genes have induced hair cell regeneration, while BDNF-loaded NPs promote spiral ganglion neuron survival. These non-viral, highly targeted approaches could pave the way for clinically viable gene therapies, overcoming the challenges of current viral vector methods and offering hope for hearing restoration.

1.4.1. Current and emerging clinical efforts. Gene therapy for hearing loss has begun to move from animal studies toward clinical trials, though most trials to date use viral vectors (Fig. 14). A notable example is the first-in-human gene therapy for congenital deafness targeting the otoferlin (OTOF) gene. OTOF mutations cause an inherited auditory neuropathy; researchers are delivering a healthy OTOF gene into children's cochleae *via* an AAV vector in an ongoing phase 1/2 trial.[83,176,177] In this approach, a single surgical injection through the round window membrane

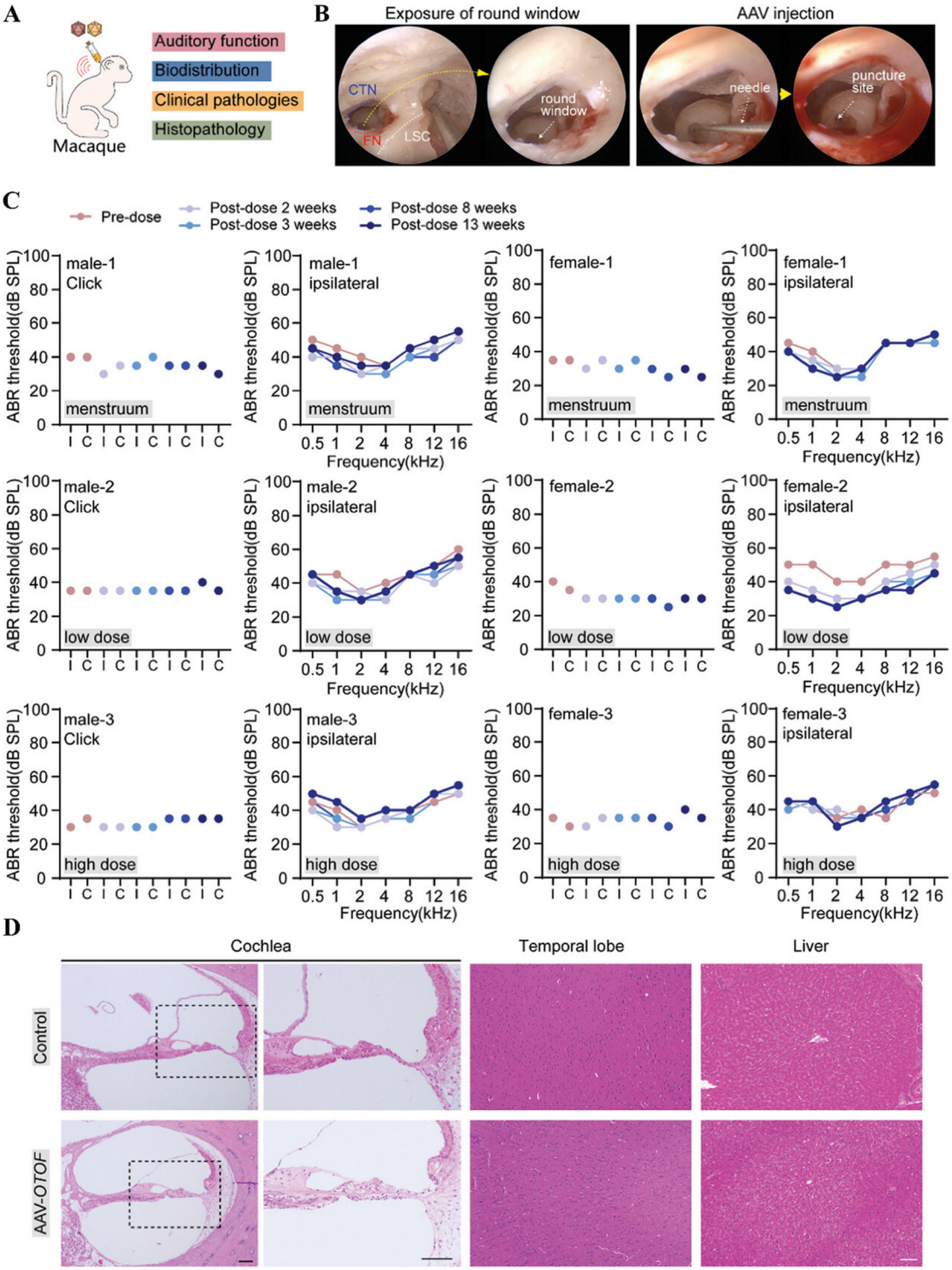

Fig. 14 AAV-OTOF demonstrates audiological and systemic safety in primate models. (A) AAV-OTOF procedure in macaques. (B) Round window exposure & injection images. (C) ABR results show safety (menstruum/low/high dose groups) (D) HE staining confirms tissue safety.[38]

introduces the vector, aiming to restore OTOF function in cochlear hair cells and enable sound signal transmission. Early reports indicate partial hearing improvements in initial patients, though full results are pending. Another prominent effort was Novartis's CGF166 trial for hair cell regeneration. This phase 1/2 trial used an adenovirus vector carrying the ATOH1 (Math1) gene to trans-differentiate inner ear supporting cells into new hair cells.[151] The vector was delivered *via* intralabyrinthine infusion (through the round window into the cochlea). While the trial's detailed outcomes have not been formally published, it

demonstrated the feasibility of surgical gene delivery in the inner ear and informed safety monitoring protocols. These viral-vector trials establish a foundation, but also highlight the limitations (*e.g.* gene size constraints and immunity) that nanotechnology may overcome in the future.

Notably, nanoparticle-based gene therapies have not yet reached human clinical trials for hearing loss as of this review. However, there are related clinical applications of nanotech in otology that pave the way. One example is a therapy for sudden sensorineural hearing loss using IGF-1 delivered in a gelatin nanoparticle-hydrogel on the round window, which advanced to clinical testing.[178] This trial showed that a neurotrophic factor could be safely delivered to the inner ear with a sustained-release system, resulting in some hearing recovery in patients. The success of such approaches supports the concept that localized nano-delivery to the cochlea is clinically achievable. We anticipate that as preclinical studies continue to demonstrate the efficacy of nanoparticle gene delivery (*e.g.* the Tmc1 CRISPR nanoparticle that preserved hearing in mice),[128] clinical translation will follow. Companies and research consortia are already exploring nanoparticle carriers for cochlear gene therapy in large animal models, aiming to initiate human trials in the coming years once safety profiles are optimized.

1.4.2. Key challenges in translation. Despite its promise, the combination of nanotechnology and gene therapy for deafness faces several hurdles before routine clinical use:

1.4.2.1. Delivery and distribution. The inner ear's anatomy and barriers make delivery challenging. The cochlea is encased in bone and protected by the blood-labyrinth barrier and tight junctions of the round window membrane (RWM). Achieving broad distribution of therapy in the cochlea without invasive surgery is difficult. Nanoparticles introduced into the middle ear must traverse the RWM or oval window to reach cochlear fluids. Small NP size helps – studies confirm that nanoparticles can diffuse through the RWM into scala tympani perilymph.[154,155,157,179] Sensorineural hearing loss, often caused by hair cell degeneration due to aging, drugs, infections, or overstimulation, is typically irreversible. The study demonstrates that delivering the Atoh1 gene *via* an adenoviral vector to nonsensory cells in the deaf cochlea induces their transdifferentiation into hair cells (Fig. 15). In experiments with guinea pigs, this approach resulted in new hair cell formation and substantial improvement in auditory brainstem response (ABR) thresholds, marking the first successful cellular and functional repair of the mature mammalian inner ear. These findings suggest a potential therapeutic strategy for hearing restoration using developmental gene expression. But many NPs still get trapped in or under the RWM if not delivered optimally. Direct injections (cochleostomy or vestibular approaches) improve delivery at the cost of an invasive procedure. Innovative catheter devices or slow-infusion pumps may be needed for precise NP delivery in patients. Additionally, once inside, nanoparticles must navigate to the target cells (hair cells in the organ of Corti, or spiral ganglion neurons). Ensuring the therapy reaches all necessary cochlear turns and cell types is an active area of research – for example, using magnetic fields to draw magnetic NPs deeper into the cochlea or engineering NPs that specifically bind hair cells. Overcoming these anatomical barriers is critical for consistent clinical outcomes.

1.4.2.2. Biocompatibility and immune response. While non-viral vectors avoid the specific immune response to viruses, any foreign material in the inner ear can cause inflammation. The inner ear has some immune privilege, but immune cells can invade in response to insults, potentially causing further damage to hearing. Nanoparticle materials must be highly biocompatible: even cationic polymers that are safe elsewhere might be ototoxic. For instance, a study found that PEI nanoparticles induced significant cochlear toxicity, negating their gene delivery benefits.[4] Surface coatings like PEG generally reduce immunogenicity, but certain components (*e.g.* cationic lipids at high dose) might trigger local inflammation or complement activation. Extensive biocompatibility testing in animal models is needed to ensure that the NP itself does not harm delicate cochlear structures or hearing function. Additionally, repeated dosing in humans will require that nanoparticles do not elicit a cumulative immune response or allergenic reaction. It's promising that in murine studies to date, appropriately designed NPs have been well-tolerated in the inner ear (*e.g.* no hair cell loss was seen with PEG-coated magnetic nanoparticles), but translating that safety to humans (with longer lifespans and larger cochleae) will be a key regulatory focus.

1.4.2.3. Regulatory and manufacturing concerns. Combining a gene therapy with a nanoparticle delivery system means navigating complex regulatory frameworks. Such a product might be regulated as a gene therapy medicinal product, requiring rigorous demonstration of safety at the genetic level (no off-target genome editing if CRISPR is used, for example) as well as at the material level (nanomaterial toxicology and degradation profile). Regulators will demand comprehensive data on where the nanoparticles travel in the body – ideally, they should remain local to the ear. Any evidence of migration to off-target organs (like brain or kidneys) would raise concern. The long-term fate of nanoparticles in the inner ear is also important: do they biodegrade and clear naturally, and how fast? Or do they persist in the cochlea? Persistence might prolong effect but could also cause chronic inflammation, so this balance must be understood. Manufacturing consistency of nanoparticle-gene products is another challenge; unlike defined viral vectors, NPs could have batch-to-batch variability in size or encapsulation efficiency. Ensuring each dose delivers the same amount of gene payload will be crucial for approval. Despite these hurdles, regulatory guidance for nanomedicine is maturing, and precedent exists in lipid nanoparticle vaccines and drugs, which should help inform the path for inner ear applications.

1.4.2.4. Ethical considerations. Treating hearing loss at the genetic level raises some ethical questions. Many candidates for gene therapy (especially for congenital deafness) are infants or

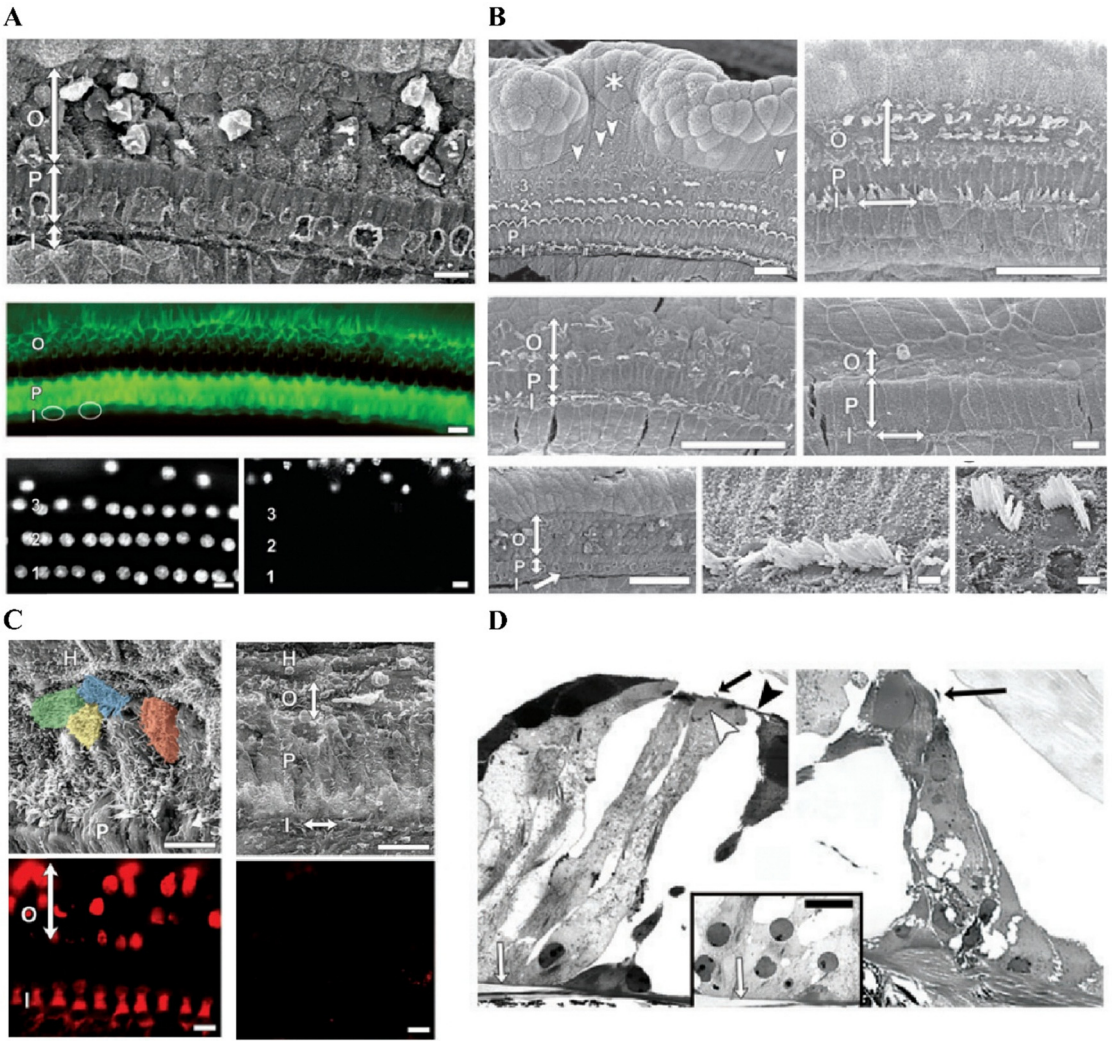

Fig. 15 Atoh1 gene therapy regenerates hair cells in deafened cochleae with varying morphology. (A) Deafened cochleae showed complete HC loss (SEM/fluorescence), with Atoh1 expression in nonsensory cells post-Atoh1 inoculation. Pillar cells survived ototoxicity. (B) Atoh1 restored HCs (IHCs/OHCs) in deafened cochleae after 2 months, though morphology varied from normal to incomplete. (C) Atoh1-treated cochleae exhibited immature stereocilia bundles and myosin VIIa+ HCs at 4–5 weeks, absent in controls. (D) Regenerated HCs showed cuticular plates, luminal projections, and basal nuclei, with some areas displaying dual nuclear layers.[24]

young children, so there are ethical implications in treating patients who cannot consent. Clinicians and families must weigh the potential lifelong benefit of hearing restoration against unknown long-term risks of a novel nanomedicine. Careful ethical oversight and informed consent (from parents/guardians) are mandatory. There is also a cultural consideration: within the Deaf community, the desirability of "curing" deafness is a nuanced topic, and introducing gene therapy could spark debate about normalizing hearing *vs.* respecting deaf identity. Transparent communication will be needed to navigate these perspectives. On the research side, if CRISPR gene editing is used, there is the ethical imperative to ensure it's strictly somatic (affecting only the ear) and cannot be passed to future generations or cause germline changes. Fortunately, delivering therapy directly to the cochlea confines the editing to the ear, mitigating germline concerns. Another consideration is equitable access: such advanced therapies might be very costly initially, raising questions of fairness in who can benefit if it reaches clinical practice.

1.5. Future outlook

The convergence of nanotechnology and gene therapy holds exciting promise for overcoming the current limitations in treating hearing loss. In the coming years, we expect to see improved nanoparticle formulations that are more efficient and even safer, moving this approach into clinical trials. One trend is the development of hybrid vectors – for example, coating AAV viral vectors with polymer or lipid nanoparticles to combine the high efficiency of viruses with the targeting and low immunogenicity of nano-carriers (early research in other fields suggests this can reduce immune detection of the virus). Another burgeoning area is exosome-based delivery, harnessing the body's own nanovesicles as gene delivery vehicles to the inner ear; these could offer natural biocompatibility and cell-specific tropism.

Moreover, refinements in delivery techniques will likely improve outcomes: image-guided injection systems, endoscopic approaches to the round window (as used in the OTOF trial), or even magnetic navigation of nanoparticles to the cochlea are being explored. As these technologies mature, it's conceivable that a patient with genetic hearing loss could receive a one-time, minimally invasive nanoparticle infusion that cures or significantly improves their hearing – a leap from managing symptoms to reversing the condition.

Encouragingly, preclinical successes are accumulating. Significant auditory recovery has already been demonstrated in animal models using non-viral nanoparticle vectors,[161] validating the concept. The gap between animal studies and human application is narrowing as delivery efficiency, targeting, and safety are optimized. Experts are optimistic that we are on the cusp of a new era where sensorineural deafness can be addressed at the molecular level. As one review noted, while challenges remain, continued innovation in nanotechnology is bringing us closer to a threshold where inner ear gene therapies become clinically viable. In summary, the fusion of nanotech and gene therapy offers a powerful platform to finally tackle hearing loss at its root cause – with precise biological fixes – and the next decade will be crucial in translating this compelling science into a real-world cure for deafness.

Author contributions

Yiwen Liu, Lin Li, Pei Huang, Dingjun Zha and Hongzhang Deng: conceptualization; writing, review and editing. Dingjun Zha and Hongzhang Deng: supervision.

Conflicts of interest

There are no conflicts to declare.

Data availability

We would like to confirm that no primary research results, software or code have been included and no new data were generated or analyzed as part of this review.

Acknowledgements

This research was supported by the National Natural Science Foundation of China projects (32171320 and 82302384) and National Key R&D Program of China (2024YFA1212500), Xidian University Specially Funded Project for Interdisciplinary Exploration (TZJH2024037).

References

1 K. K. Brewster, J. S. Golub and B. R. Rutherford, Neural circuits and behavioral pathways linking hearing loss to affective dysregulation in older adults, *Nat. Aging*, 2021, **1**(5), 422–429.
2 A. M. H. Korver, R. J. H. Smith, G. Van Camp, M. R. Schleiss, M. A. K. Bitner-Glindzicz, L. R. Lustig, S.-I. Usami and A. N. Boudewyns, Congenital hearing loss, *Nat. Rev. Dis. Primers*, 2017, **3**(1), 16094.
3 U. Müller and P. G. Barr-Gillespie, New treatment options for hearing loss, *Nat. Rev. Drug Discovery*, 2015, **14**(5), 346–365.
4 C. Valentini, B. Szeto, J. W. Kysar and A. K. Lalwani, Inner Ear Gene Delivery: Vectors and Routes, *Hearing Balance Commun.*, 2020, **18**(4), 278–285.
5 F. Tan, C. Chu, J. Qi, W. Li, D. You, K. Li, X. Chen, W. Zhao, C. Cheng, X. Liu, Y. Qiao, B. Su, S. He, C. Zhong, H. Li, R. Chai and G. Zhong, AAV-ie enables safe and efficient gene transfer to inner ear cells, *Nat. Commun.*, 2019, **10**(1), 3733.
6 X. Ma, L.-N. Chen, M. Liao, L. Zhang, K. Xi, J. Guo, C. Shen, D.-D. Shen, P. Cai, Q. Shen, J. Qi, H. Zhang, S.-K. Zang, Y.-J. Dong, L. Miao, J. Qin, S.-Y. Ji, Y. Li, J. Liu, C. Mao, Y. Zhang and R. Chai, Molecular insights into the activation mechanism of GPR156 in maintaining auditory function, *Nat. Commun.*, 2024, **15**(1), 10601.
7 J. Lv, H. Wang, X. Cheng, Y. Chen, D. Wang, L. Zhang, Q. Cao, H. Tang, S. Hu, K. Gao, M. Xun, J. Wang, Z. Wang, B. Zhu, C. Cui, Z. Gao, L. Guo, S. Yu, L. Jiang, Y. Yin, J. Zhang, B. Chen, W. Wang, R. Chai, Z.-Y. Chen, H. Li and Y. Shu, AAV1-hOTOF gene therapy for autosomal recessive deafness 9: a single-arm trial, *Lancet*, 2024, **403**(10441), 2317–2325.
8 Z. Liu, H. Zhang, G. Hong, X. Bi, J. Hu, T. Zhang, Y. An, N. Guo, F. Dong, Y. Xiao, W. Li, X. Zhao, B. Chu, S. Guo, X. Zhang, R. Chai and X. Fu, Inhibition of Gpx4-mediated ferroptosis alleviates cisplatin-induced hearing loss in C57BL/6 mice, *Mol. Ther.*, 2024, **32**(5), 1387–1406.
9 G. M. Clark, The multichannel cochlear implant for severe-to-profound hearing loss, *Nat. Med.*, 2013, **19**(10), 1236–1239.
10 S. L. Cushing, B. C. Papsin, J. A. Rutka, A. L. James and K. A. Gordon, Evidence of Vestibular and Balance Dysfunction in Children With Profound Sensorineural Hearing Loss Using Cochlear Implants, *Laryngoscope*, 2008, **118**(10), 1814–1823.
11 D. R. Moore and R. V. Shannon, Beyond cochlear implants: awakening the deafened brain, *Nat. Neurosci.*, 2009, **12**(6), 686–691.
12 B. McMurray, F. X. Smith, M. Huffman, K. Rooff, J. B. Muegge, C. Jeppsen, E. Kutlu and S. Colby, Underlying dimensions of real-time word recognition in cochlear implant users, *Nat. Commun.*, 2024, **15**(1), 7382.
13 C. Petit, C. Bonnet and S. Safieddine, Deafness: from genetic architecture to gene therapy, *Nat. Rev. Genet.*, 2023, **24**(10), 665–686.
14 M. Duan, F. Venail, N. Spencer and M. Mezzina, Treatment of peripheral sensorineural hearing loss: gene therapy, *Gene Ther.*, 2004, **11**(1), S51–S56.
15 J. Lye, D. S. Delaney, F. K. Leith, V. S. Sardesai, S. McLenachan, F. K. Chen, M. D. Atlas and E. Y. M. Wong,

Recent Therapeutic Progress and Future Perspectives for the Treatment of Hearing Loss, *Biomedicines*, 2023, **11**(12), 3347.
16 N. Ding, S. Lee, M. Lieber-Kotz, J. Yang and X. Gao, Advances in genome editing for genetic hearing loss, *Adv. Drug Delivery Rev.*, 2021, **168**, 118–133.
17 H. Liu, J. Hao and K. S. Li, Current strategies for drug delivery to the inner ear, *Acta Pharm. Sin. B*, 2013, **3**(2), 86–96.
18 S. Nyberg, N. J. Abbott, X. Shi, P. S. Steyger and A. Dabdoub, Delivery of therapeutics to the inner ear: The challenge of the blood-labyrinth barrier, *Sci. Transl. Med.*, 2019, **11**(482), eaao0935.
19 X. An and D. Zha, Development of Nanoparticle Drug-Delivery Systems for The Inner Ear, *Nanomedicine*, 2020, **15**(20), 1981–1993.
20 Z. Li, Y. Gao, X. Chen, L. Xu, Z. Li and R. Chai, Study on Recovery Strategy of Hearing Loss & SGN Regeneration Under Physical Regulation, *Adv. Sci.*, 2025, **12**(5), 2410919.
21 M. S. Hildebrand, S. S. Newton, S. P. Gubbels, A. M. Sheffield, A. Kochhar, M. G. de Silva, H.-H. M. Dahl, S. D. Rose, M. A. Behlke and R. J. H. Smith, Advances in Molecular and Cellular Therapies for Hearing Loss, *Mol. Ther.*, 2008, **16**(2), 224–236.
22 K. Xu, B. Xu, J. Gu, X. Wang, D. Yu and Y. Chen, Intrinsic mechanism and pharmacologic treatments of noise-induced hearing loss, *Theranostics*, 2023, **13**(11), 3524–3549.
23 Q. Yu, S. Liu, R. Guo, K. Chen, Y. Li, D. Jiang, S. Gong, L. Yin and K. Liu, Complete Restoration of Hearing Loss and Cochlear Synaptopathy *via* Minimally Invasive, Single-Dose, and Controllable Middle Ear Delivery of Brain-Derived Neurotrophic Factor–Poly(dl-lactic acid-*co*-glycolic acid)-Loaded Hydrogel, *ACS Nano*, 2024, **18**(8), 6298–6313.
24 M. Izumikawa, R. Minoda, K. Kawamoto, K. A. Abrashkin, D. L. Swiderski, D. F. Dolan, D. E. Brough and Y. Raphael, Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals, *Nat. Med.*, 2005, **11**(3), 271–276.
25 X. Gao, Y. Tao, V. Lamas, M. Huang, W.-H. Yeh, B. Pan, Y.-J. Hu, J. H. Hu, D. B. Thompson, Y. Shu, Y. Li, H. Wang, S. Yang, Q. Xu, D. B. Polley, M. C. Liberman, W.-J. Kong, J. R. Holt, Z.-Y. Chen and D. R. Liu, Treatment of autosomal dominant hearing loss by in vivo delivery of genome editing agents, *Nature*, 2018, **553**(7687), 217–221.
26 X. Lin, W. Tang, S. Ahmad, J. Lu, C. C. Colby, J. Zhu and Q. Yu, Applications of targeted gene capture and next-generation sequencing technologies in studies of human deafness and other genetic disabilities, *Hear. Res.*, 2012, **288**(1), 67–76.
27 J. W. Schott, P. Huang, M. Morgan, J. Nelson-Brantley, A. Koehler, B. Renslo, H. Büning, A. Warnecke, A. Schambach and H. Staecker, Third-generation lentiviral gene therapy rescues function in a mouse model of Usher 1B, *Mol. Ther.*, 2023, **31**(12), 3502–3519.
28 Y. Xue, Y. Tao, X. Wang, X. Wang, Y. Shu, Y. Liu, W. Kang, S. Chen, Z. Cheng, B. Yan, Y. Xie, L. Bi, H. Jia, J. Li, Q. Xiao, L. Chen, X. Yao, L. Shi, H. Yang and H. Wu, RNA base editing therapy cures hearing loss induced by OTOF gene mutation, *Mol. Ther.*, 2023, **31**(12), 3520–3530.
29 J. Qi, L. Xu, F.-G. Zeng and R. Chai, OTOF-related gene therapy: a new way but a long road ahead, *Lancet*, 2025, **405**(10481), 777–779.
30 E. N. Liao, E. Taketa, N. I. Mohamad and D. K. Chan, Outcomes of Gene Panel Testing for Sensorineural Hearing Loss in a Diverse Patient Cohort, *JAMA Network Open*, 2022, **5**(9), e2233441.
31 J. Nie, Y. Ueda, A. J. Solivais and E. Hashino, CHD7 regulates otic lineage specification and hair cell differentiation in human inner ear organoids, *Nat. Commun.*, 2022, **13**(1), 7053.
32 E. J. Simons and I. Trapani, The Opportunities and Challenges of Gene Therapy for Treatment of Inherited Forms of Vision and Hearing Loss, *Hum. Gene Ther.*, 2023, **34**(17–18), 808–820.
33 S. A. S. Hussain, M. H. Ali, M. H. Imtiaz and M. Al Hasibuzzaman, Breaking the silence: gene therapy offers hope for OTOF-mediated hearing loss, editorial, *Ann. Med. Surg.*, 2024, **86**(9), 4950–4951.
34 J. V. Brigande, Otoferlin gene therapy restores hearing in deaf children, *Mol. Ther.*, 2024, **32**(4), 859–860.
35 A. R. Saidia, J. Ruel, A. Bahloul, B. Chaix, F. Venail and J. Wang, Current Advances in Gene Therapies of Genetic Auditory Neuropathy Spectrum Disorder, *J. Clin. Med.*, 2023, **12**(3), 738.
36 M. J. Klimara and R. J. H. Smith, Advances in cochlear gene therapies, *Curr. Opin. Pediatr.*, 2023, **35**(6), 631–640.
37 J. Qi, L. Zhang, F. Tan, Y. Zhang, Y. Zhou, Z. Zhang, H. Wang, C. Yu, L. Jiang, J. Liu, T. Chen, L. Wu, S. Zhang, S. Sun, S. Sun, L. Lu, Q. Wang and R. Chai, Preclinical Efficacy And Safety Evaluation of AAV-OTOF in DFNB9 Mouse Model And Nonhuman Primate, *Adv. Sci.*, 2024, **11**(3), 2306201.
38 J. Qi, F. Tan, L. Zhang, L. Lu, S. Zhang, Y. Zhai, Y. Lu, X. Qian, W. Dong, Y. Zhou, Z. Zhang, X. Yang, L. Jiang, C. Yu, J. Liu, T. Chen, L. Wu, C. Tan, S. Sun, H. Song, Y. Shu, L. Xu, X. Gao, H. Li and R. Chai, AAV-Mediated Gene Therapy Restores Hearing in Patients with DFNB9 Deafness, *Adv. Sci.*, 2024, **11**(11), 2306788.
39 W. Cui, M. Guo, D. Liu, P. Xiao, C. Yang, H. Huang, C. Liang, Y. Yang, X. Fu, Y. Zhang, J. Liu, S. Shi, J. Cong, Z. Han, Y. Xu, L. Du, C. Yin, Y. Zhang, J. Sun, W. Gu, R. Chai, S. Zhu and B. Chu, Gut microbial metabolite facilitates colorectal cancer development via ferroptosis inhibition, *Nat. Cell Biol.*, 2024, **26**(1), 124–137.
40 H. Al-Moyed, A. P. Cepeda, S. Jung, T. Moser, S. Kügler and E. Reisinger, A dual-AAV approach restores fast exocytosis and partially rescues auditory function in deaf otoferlin knock-out mice, *EMBO Mol. Med.*, 2019, **11**(1), e9396.
41 T. Foster, M. Lewkowicz, C. Quintas, C. M. Ionescu, M. Jones, S. R. Wagle, B. Kovacevic, E. Y. M. Wong, A. Mooranian and H. Al-Salami, Novel Nanoencapsulation Technology and its Potential Role in Bile Acid-Based

Targeted Gene Delivery to the Inner Ear, *Small*, 2023, **19**(8), 2204986.
42 A. Kashizadeh, C. Pastras, N. Rabiee, M. Mohseni-Dargah, P. Mukherjee and M. Asadnia, Potential nanotechnology-based diagnostic and therapeutic approaches for Meniere's disease, *Nanomedicine*, 2022, **46**, 102599.
43 X. Xu, S. Wang, Y. Shu and H. Zhang, Nanoparticle-based inner ear delivery systems for the treatment of hearing loss, *Smart Mater. Med.*, 2021, **2**, 350–353.
44 V. Leso, F. Luca, E. M. Luigia, R. Rosaria and I. Iavicoli, Opportunities and Challenging Issues of Nanomaterials in Otological Fields: an Occupational Health Perspective, *Nanomedicine*, 2019, **14**(19), 2613–2629.
45 B. Acharya, A. Behera, S. Behera and S. Moharana, Recent Advances in Nanotechnology-Based Drug Delivery Systems for the Diagnosis and Treatment of Reproductive Disorders, *ACS Appl. Bio Mater.*, 2024, **7**(3), 1336–1361.
46 M. DeLuca, Z. Shi, C. E. Castro and G. Arya, Dynamic DNA nanotechnology: toward functional nanoscale devices, *Nanoscale Horiz.*, 2020, **5**(2), 182–201.
47 C. O. Franck, A. Bistrovic Popov, I. Ahmed, R. E. Hewitt, L. Franslau, P. Tyagi and L. Fruk, A catch-and-release nano-based gene delivery system, *Nanoscale Horiz.*, 2023, **8**(11), 1588–1594.
48 J. Li and H. Wang, Selective organ targeting nanoparticles: from design to clinical translation, *Nanoscale Horiz.*, 2023, **8**(9), 1155–1173.
49 J. Joo, E. J. Kwon, J. Kang, M. Skalak, E. J. Anglin, A. P. Mann, E. Ruoslahti, S. N. Bhatia and M. J. Sailor, Porous silicon–graphene oxide core–shell nanoparticles for targeted delivery of siRNA to the injured brain, *Nanoscale Horiz.*, 2016, **1**(5), 407–414.
50 S. Chung, C. M. Lee and M. Zhang, Advances in nanoparticle-based mRNA delivery for liver cancer and liver-associated infectious diseases, *Nanoscale Horiz.*, 2023, **8**(1), 10–28.
51 Y. Chien, Y.-J. Hsiao, S.-J. Chou, T.-Y. Lin, A. A. Yarmishyn, W.-Y. Lai, M.-S. Lee, Y.-Y. Lin, T.-W. Lin, D.-K. Hwang, T.-C. Lin, S.-H. Chiou, S.-J. Chen and Y.-P. Yang, Nanoparticles-mediated CRISPR-Cas9 gene therapy in inherited retinal diseases: applications, challenges, and emerging opportunities, *J. Nanobiotechnol.*, 2022, **20**(1), 511.
52 T. Foster, L. Patrick, I. C. Mihaela, W. S. Raj, K. Bozica, M. Armin and H. Al-Salami, Exploring delivery systems for targeted nanotechnology-based gene therapy in the inner ear, *Ther. Delivery*, 2024, **15**(10), 801–818.
53 H. Yin, K. J. Kauffman and D. G. Anderson, Delivery technologies for genome editing, *Nat. Rev. Drug Discovery*, 2017, **16**(6), 387–399.
54 Y. Xu, Z. Bei, M. Li, K. Qiu, J. Ren, B. Chu, Y. Zhao and Z. Qian, Biomaterials for non-invasive trans-tympanic drug delivery: requirements, recent advances and perspectives, *J. Mater. Chem. B*, 2024, **12**(32), 7787–7813.
55 Y. Xu, T. Fourniols, Y. Labrak, V. Préat, A. Beloqui and A. des Rieux, Surface Modification of Lipid-Based Nanoparticles, *ACS Nano*, 2022, **16**(5), 7168–7196.
56 F. Zheng and J. Zuo, Cochlear hair cell regeneration after noise-induced hearing loss: Does regeneration follow development?, *Hear. Res.*, 2017, **349**, 182–196.
57 Y. Lee, M. Jeong, J. Park, H. Jung and H. Lee, Immunogenicity of lipid nanoparticles and its impact on the efficacy of mRNA vaccines and therapeutics, *Exp. Mol. Med.*, 2023, **55**(10), 2085–2096.
58 Y. Liu, Q. Liu, B. Zhang, S. Chen, Y. Shen, Z. Li, J. Zhang, Y. Yang, M. Li and Y. Wang, Generation of tolerogenic antigen-presenting cells in vivo via the delivery of mRNA encoding PDL1 within lipid nanoparticles, *Nat. Biomed. Eng.*, 2025, DOI: **10.1038/s41551-025-01373-0**.
59 W. Ho, M. Gao, F. Li, Z. Li, X.-Q. Zhang and X. Xu, Next-Generation Vaccines: Nanoparticle-Mediated DNA and mRNA Delivery, *Adv. Healthcare Mater.*, 2021, **10**(8), 2001812.
60 Z. Qin, M. Wood and J. J. Rosowski, Measurement of conductive hearing loss in mice, *Hear. Res.*, 2010, **263**(1), 93–103.
61 C. Clarkson, F. M. Antunes and M. E. Rubio, Conductive Hearing Loss Has Long-Lasting Structural and Molecular Effects on Presynaptic and Postsynaptic Structures of Auditory Nerve Synapses in the Cochlear Nucleus, *J. Neurosci.*, 2016, **36**(39), 10214.
62 S. M. Vlajkovic and P. R. Thorne, Molecular Mechanisms of Sensorineural Hearing Loss and Development of Inner Ear Therapeutics, *Int. J. Mol. Sci.*, 2021, **22**(11), 5647.
63 S. G. Kujawa and M. C. Liberman, Synaptopathy in the noise-exposed and aging cochlea: Primary neural degeneration in acquired sensorineural hearing loss, *Hear. Res.*, 2015, **330**, 191–199.
64 S. N. Merchant and J. J. Rosowski, Conductive Hearing Loss Caused by Third-Window Lesions of the Inner Ear, *Otol. Neurotol.*, 2008, **29**(3), 282–289.
65 G. Paludetti, G. Conti, D. I. N. W, D. E. C. E, R. Rolesi, P. M. Picciotti and A. R. Fetoni, Infant hearing loss: from diagnosis to therapy Official Report of XXI Conference of Italian Society of Pediatric Otorhinolaryngology, *Acta Otorhinolaryngol. Ital.*, 2012, **32**(6), 347–370.
66 C. B. Paik, M. Pei and J. S. Oghalai, Review of blast noise and the auditory system, *Hear. Res.*, 2022, **425**, 108459.
67 L. X. Zhong, S. Kun, Q. Jing, C. Jing and Y. Denise, Non-Syndromic Hearing Loss and High-Throughput Strategies to Decipher Its Genetic Heterogeneity, *J. Otol.*, 2013, **8**(1), 6–24.
68 A. Larem, Z. Altamimi and A. Aljariri, Sensorineural Hearing Loss (SNHL), in *Textbook of Clinical Otolaryngology*, ed. A. Al-Qahtani, H. Haidar and A. Larem, Springer International Publishing, Cham, 2021, pp. 111–119.
69 A. G. M. Schilder, S. Wolpert, S. Saeed, L. M. Middelink, A. S. B. Edge, H. Blackshaw, A. Schilder, L. Middelink, A. Edge, A. Bibas, E. Arram, A. Bilhet, H. Cooper, E. Dalhoff, F. van Diggelen, R. J. Rutten, H. van Es, K. Hojgaard, E. Iliadou, O. Yildirim, S. Khalil, D. Kikidis, H. Lowenheim, N. Markatos, M. Mueller, T. Schade-Mann, F. Schneider, K. Vardonikolaki, A. Wilke, K. Pastiadis, A. G. Bibas and R. Consortium, A phase I/IIa safety and

efficacy trial of intratympanic gamma-secretase inhibitor as a regenerative drug treatment for sensorineural hearing loss, *Nat. Commun.*, 2024, **15**(1), 1896.

70 W. Xiao, K. He, C. Yu, Z. Zhou, L. Xia, S. Xie, H. Li, M. Zhang, Z. Zhang, P. Luo, L. Wen and G. Chen, Space Station-like Composite Nanoparticles for Co-Delivery of Multiple Natural Compounds from Chinese Medicine and Hydrogen in Combating Sensorineural Hearing Loss, *Mol. Pharm.*, 2023, **20**(8), 3987–4006.

71 C. He, H. Gai, W. Zhao, H. Zhang, L. Lai, C. Ding, L. Chen and J. Ding, Advances in the Study of Etiology and Molecular Mechanisms of Sensorineural Hearing Loss, *Cell Biochem. Biophys.*, 2024, **82**(3), 1721–1734.

72 M. C. Liberman and S. G. Kujawa, Cochlear synaptopathy in acquired sensorineural hearing loss: Manifestations and mechanisms, *Hear. Res.*, 2017, **349**, 138–147.

73 T. Moser and A. Starr, Auditory neuropathy—neural and synaptic mechanisms, *Nat. Rev. Neurol.*, 2016, **12**(3), 135–149.

74 G. Rance and A. Starr, Pathophysiological mechanisms and functional hearing consequences of auditory neuropathy, *Brain*, 2015, **138**(11), 3141–3158.

75 G. R. Merchant and S. T. Neely, Conductive Hearing Loss Estimated From Wideband Acoustic Immittance Measurements in Ears With Otitis Media With Effusion, *Ear Hear.*, 2023, **44**(4), 721–731.

76 M. Batuk, B. Gündüz and A. R. Fetoni, Audiological Approach in Bone-Anchored Hearing Aid Applications, in *Neurotology Updates*, ed. M. T. Kalcioglu, N. Bayar Muluk and H. A. Jenkins, Springer Nature Switzerland, Cham, 2024, pp. 437–460.

77 T. Kleinjung and A. Londero, Conductive and Sensorineural Hearing Loss, in *Textbook of Tinnitus*, ed. W. Schlee, B. Langguth, D. De Ridder, S. Vanneste, T. Kleinjung and A. R. Møller, Springer International Publishing, Cham, 2024, pp. 385–398.

78 M. Subramanian, A. Chawla, K. Chokkappan, T. Lim, J. N. Shenoy and W. Chin Guan Peh, High-Resolution Computed Tomography Imaging in Conductive Hearing Loss: What to Look for?, *Curr. Probl. Diagn. Radiol.*, 2018, **47**(2), 119–124.

79 P. R. Hill-Feltham, M. L. Johansson, W. E. Hodgetts, A. V. Ostevik, B. J. McKinnon, M. Peter, S. Ravi, W. Tracy and J. R. Tysome, Hearing outcome measures for conductive and mixed hearing loss treatment in adults: a scoping review, *Int. J. Audiol.*, 2021, **60**(4), 239–245.

80 M. Winiger Allison, M. Alexander Joshua and O. Diefendorf Allan, Minimal Hearing Loss: From a Failure-Based Approach to Evidence-Based Practice, *Am. J. Audiol.*, 2016, **25**(3), 232–245.

81 L. Jiang, D. Wang, Y. He and Y. Shu, Advances in gene therapy hold promise for treating hereditary hearing loss, *Mol. Ther.*, 2023, **31**(4), 934–950.

82 X. Gu, X. Hu, D. Wang, Z. Xu, F. Wang, D. Li, G. L. Li, H. Yang, H. Li, E. Zuo and Y. Shu, Treatment of autosomal recessive hearing loss via in vivo CRISPR/Cas9-mediated optimized homology-directed repair in mice, *Cell Res.*, 2022, **32**(7), 699–702.

83 L. Zhang, F. Tan, J. Qi, Y. Lu, X. Wang, X. Yang, X. Chen, X. Zhang, J. Fan, Y. Zhou, L. Peng, N. Li, L. Xu, S. Yang and R. Chai, AAV-mediated Gene Therapy for Hereditary Deafness: Progress and Perspectives, *Adv. Sci.*, 2024, **11**(47), 2402166.

84 F. Tan, Y. Dong, J. Qi, W. Yu and R. Chai, Artificial Intelligence-Based Approaches for AAV Vector Engineering, *Adv. Sci.*, 2025, **12**(9), 2411062.

85 Q. Sun, L. Zhang, T. Chen, N. Li, F. Tan, X. Gu, Y. Zhou, Z. Zhang, Y. Lu, J. Lu, X. Qian, B. Guan, J. Qi, F. Ye and R. Chai, AAV-mediated Gpm6b expression supports hair cell reprogramming, *Cell Prolif.*, 2024, **57**(7), e13620.

86 Q. Sun, F. Tan, L. Zhang, Y. Lu, H. Wei, N. Li, L. Jiang, Y. Zhou, T. Chen, L. Lu, G.-L. Li, J. Qi, S. Yang and R. Chai, Combined AAV-mediated specific Gjb2 expression restores hearing in DFNB1 mouse models, *Mol. Ther.*, 2025, **33**(7), 3006–3021.

87 N. Li, F. Tan, L. Zhang, X. Ding, Q. Sun, M. Wang, Z. Zhang, Y. Lu, Y. Zhou, X. Qian, F. Ye, J. Qi and R. Chai, AAV-Sparcl1 promotes hair cell regeneration by increasing supporting cell plasticity, *Mol. Ther.*, 2025, **33**(7), 3022–3035.

88 C. Zhao, C. Wang, W. Shan, W. Wang and H. Deng, Fusogenic Lipid Nanovesicle for Biomacromolecular Delivery, *Nano Lett.*, 2024, **24**(28), 8609–8618.

89 P. Huang, H. Deng, C. Wang, Y. Zhou and X. Chen, Cellular Trafficking of Nanotechnology-Mediated mRNA Delivery, *Adv. Mater.*, 2024, **36**(13), 2307822.

90 C. Wang, C. Zhao, W. Wang, X. Liu and H. Deng, Biomimetic noncationic lipid nanoparticles for mRNA delivery, *Proc. Natl. Acad. Sci. U. S. A.*, 2023, **120**(51), e2311276120.

91 P. Huang, L. Jiang, H. Pan, L. Ding, B. Zhou, M. Zhao, J. Zou, B. Li, M. Qi, H. Deng, Y. Zhou and X. Chen, An Integrated Polymeric mRNA Vaccine without Inflammation Side Effects for Cellular Immunity Mediated Cancer Therapy, *Adv. Mater.*, 2023, **35**(3), 2207471.

92 Q. Zheng, T. Ma and M. Wang, Unleashing the Power of Proenzyme Delivery for Targeted Therapeutic Applications Using Biodegradable Lipid Nanoparticles, *Acc. Chem. Res.*, 2024, **57**(2), 208–221.

93 S. Zhao, K. Gao, H. Han, M. Stenzel, B. Yin, H. Song, A. Lawanprasert, J. E. Nielsen, R. Sharma, O. H. Arogundade, S. Pimcharoen, Y.-J. Chen, A. Paul, J. Tuma, M. G. Collins, Y. Wyle, M. G. Cranick, B. W. Burgstone, B. S. Perez, A. E. Barron, A. M. Smith, H. Y. Lee, A. Wang and N. Murthy, Acid-degradable lipid nanoparticles enhance the delivery of mRNA, *Nat. Nanotechnol.*, 2024, **19**(11), 1702–1711.

94 S. Liu, Y. Wen, X. Shan, X. Ma, C. Yang, X. Cheng, Y. Zhao, J. Li, S. Mi, H. Huo, W. Li, Z. Jiang, Y. Li, J. Lin, L. Miao and X. Lu, Charge-assisted stabilization of lipid nanoparticles enables inhaled mRNA delivery for mucosal vaccination, *Nat. Commun.*, 2024, **15**(1), 9471.

95 P. R. Cullis and P. L. Felgner, The 60-year evolution of lipid nanoparticles for nucleic acid delivery, *Nat. Rev. Drug Discovery*, 2024, **23**(9), 709–722.

96 A. E. I. Hamouda, J. Filtjens, E. Brabants, D. Kancheva, A. Debraekeleer, J. Brughmans, L. Jacobs, P. M. R. Bardet, E. Knetemann, P. Lefesvre, L. Allonsius, M. Gontsarik, I. Varela, M. Crabbé, E. J. Clappaert, F. Cappellesso, A. A. Caro, A. Gordún Peiró, L. Fredericq, E. Hadadi, M. Estapé Senti, R. Schiffelers, L. A. van Grunsven, F. Aboubakar Nana, B. G. De Geest, S. Deschoemaeker, S. De Koker, F. Lambolez and D. Laoui, Intratumoral delivery of lipid nanoparticle-formulated mRNA encoding IL-21, IL-7, and 4-1BBL induces systemic anti-tumor immunity, *Nat. Commun.*, 2024, **15**(1), 10635.

97 P. Huang, H. Deng, Y. Zhou and X. Chen, The roles of polymers in mRNA delivery, *Matter*, 2022, **5**(6), 1670–1699.

98 A. Ishaqat and A. Herrmann, Polymers Strive for Accuracy: From Sequence-Defined Polymers to mRNA Vaccines against COVID-19 and Polymers in Nucleic Acid Therapeutics, *J. Am. Chem. Soc.*, 2021, **143**(49), 20529–20545.

99 X. Huang, N. Kong, X. Zhang, Y. Cao, R. Langer and W. Tao, The landscape of mRNA nanomedicine, *Nat. Med.*, 2022, **28**(11), 2273–2287.

100 L. Rotolo, D. Vanover, N. C. Bruno, H. E. Peck, C. Zurla, J. Murray, R. K. Noel, L. O'Farrell, M. Araínga, N. Orr-Burks, J. Y. Joo, L. C. S. Chaves, Y. Jung, J. Beyersdorf, S. Gumber, R. Guerrero-Ferreira, S. Cornejo, M. Thoresen, A. K. Olivier, K. M. Kuo, J. C. Gumbart, A. R. Woolums, F. Villinger, E. R. Lafontaine, R. J. Hogan, M. G. Finn and P. J. Santangelo, Species-agnostic polymeric formulations for inhalable messenger RNA delivery to the lung, *Nat. Mater.*, 2023, **22**(3), 369–379.

101 E. Ben-Akiva, J. Karlsson, S. Hemmati, H. Yu, S. Y. Tzeng, D. M. Pardoll and J. J. Green, Biodegradable lipophilic polymeric mRNA nanoparticles for ligand-free targeting of splenic dendritic cells for cancer vaccination, *Proc. Natl. Acad. Sci. U. S. A.*, 2023, **120**(26), e2301606120.

102 A. vander Straeten, M. Sarmadi, J. L. Daristotle, M. Kanelli, L. H. Tostanoski, J. Collins, A. Pardeshi, J. Han, D. Varshney, B. Eshaghi, J. Garcia, T. A. Forster, G. Li, N. Menon, S. L. Pyon, L. Zhang, C. Jacob-Dolan, O. C. Powers, K. Hall, S. K. Alsaiari, M. Wolf, M. W. Tibbitt, R. Farra, D. H. Barouch, R. Langer and A. Jaklenec, A microneedle vaccine printer for thermostable COVID-19 mRNA vaccines, *Nat. Biotechnol.*, 2024, **42**(3), 510–517.

103 J. Casper, S. H. Schenk, E. Parhizkar, P. Detampel, A. Dehshahri and J. Huwyler, Polyethylenimine (PEI) in gene therapy: Current status and clinical applications, *J. Controlled Release*, 2023, **362**, 667–691.

104 J. Wang, H. Wang, P. Zhao, Z. Chen and Q. Lin, Hyperbranched-star PEI-g-PEG as a nonviral vector with efficient uptake and hypotoxicity for retinoblastoma gene therapy application, *Colloid Interface Sci. Commun.*, 2022, **50**, 100647.

105 T. Vasiliu, B. F. Craciun, A. Neamtu, L. Clima, D. L. Isac, S. S. Maier, M. Pinteala, F. Mocci and A. Laaksonen, In silico study of PEI-PEG-squalene-dsDNA polyplex formation: the delicate role of the PEG length in the binding of PEI to DNA, *Biomater. Sci.*, 2021, **9**(19), 6623–6640.

106 S. K. Samal, M. Dash, S. Van Vlierberghe, D. L. Kaplan, E. Chiellini, C. van Blitterswijk, L. Moroni and P. Dubruel, Cationic polymers and their therapeutic potential, *Chem. Soc. Rev.*, 2012, **41**(21), 7147–7194.

107 H. Zhou, X. Ma, Y. Liu, L. Dong, Y. Luo, G. Zhu, X. Qian, J. Chen, L. Lu, J. Wang and X. Gao, Linear polyethylenimine-plasmid DNA nanoparticles are ototoxic to the cultured sensory epithelium of neonatal mice, *Mol. Med. Rep.*, 2015, **11**(6), 4381–4388.

108 G.-G. Chen, M. Mao, L.-Z. Qiu and Q.-M. Liu, Gene transfection mediated by polyethyleneimine-polyethylene glycol nanocarrier prevents cisplatin-induced spiral ganglion cell damage, *Neural Regener. Res.*, 2015, **10**(3), 425–431.

109 F. Chen, Q. Jiang, B. Xu, Y. Huang, K. Xu, X. Xu, D. Yu, Y. Chen and X. Wang, Ototoxicity-Alleviating and Cytoprotective Allomelanin Nanomedicine for Efficient Sensorineural Hearing Loss Treatment, *ACS Nano*, 2024, **18**(38), 26401–26418.

110 P.-W. Ma, P.-H. Lu, H. Yuan, J.-W. Chen, W. Gao, Y.-Q. Lun, J.-N. Guo, X.-R. Ding, R. Liang, S.-Y. Li, Z. Wang, W.-L. Wang and L.-J. Lu, Ferrostatin-1-loaded thermosensitive nanodelivery system for noise-induced hearing loss treatment, *Chem. Eng. J.*, 2023, **476**, 146584.

111 M. M. Goyal, N. J. Zhou, P. F. Y. Vincent, E. S. Hoffman, S. Goel, C. Wang and D. Q. Sun, Rationally Designed Magnetic Nanoparticles for Cochlear Drug Delivery: Synthesis, Characterization, and In Vitro Biocompatibility in a Murine Model, *Otol. Neurotol. Open*, 2022, **2**(3), e013.

112 A. Kumar, H. Ma, X. Zhang, K. Huang, S. Jin, J. Liu, T. Wei, W. Cao, G. Zou and X.-J. Liang, Gold nanoparticles functionalized with therapeutic and targeted peptides for cancer treatment, *Biomaterials*, 2012, **33**(4), 1180–1189.

113 J. W. Lee, S.-R. Choi and J. H. Heo, Simultaneous Stabilization and Functionalization of Gold Nanoparticles *via* Biomolecule Conjugation: Progress and Perspectives, *ACS Appl. Mater. Interfaces*, 2021, **13**(36), 42311–42328.

114 R. H. Huang, N. Nayeem, Y. He, J. Morales, D. Graham, R. Klajn, M. Contel, S. O'Brien and R. V. Ulijn, Self-Complementary Zwitterionic Peptides Direct Nanoparticle Assembly and Enable Enzymatic Selection of Endocytic Pathways, *Adv. Mater.*, 2022, **34**(1), 2104962.

115 D. Hao, X. Zhang, R. Su, Y. Wang and W. Qi, Biomolecule-protected gold nanoclusters: synthesis and biomedical applications, *J. Mater. Chem. B*, 2023, **11**(23), 5051–5070.

116 A. V. Ramsey, A. J. Bischoff and M. B. Francis, Enzyme Activated Gold Nanoparticles for Versatile Site-Selective Bioconjugation, *J. Am. Chem. Soc.*, 2021, **143**(19), 7342–7350.

117 M. J. Martín, G. Spitzmaul and V. Lassalle, Novel Insights and Perspectives for the Diagnosis and Treatment of Hearing Loss through the Implementation of Magnetic Nanotheranostics, *ChemMedChem*, 2022, **17**(5), e202100685.

118 K. Talaśka, D. Wojtkowiak, D. Wilczyński and A. Ferreira, Computational methodology for drug delivery to the inner ear using magnetic nanoparticle aggregates, *Comput. Methods Programs Biomed.*, 2022, **221**, 106860.

119 B. Rezaei, A. Harun, X. Wu, P. R. Iyer, S. Mostufa, S. Ciannella, I. H. Karampelas, J. Chalmers, I. Srivastava, J. Gómez-Pastora and K. Wu, Effect of Polymer and Cell Membrane Coatings on Theranostic Applications of Nanoparticles: A Review, *Adv. Healthcare Mater.*, 2024, **13**(26), 2401213.
120 R. Bushra, M. Ahmad, K. Alam, F. Seidi, Qurtulen, S. Shakeel, J. Song, Y. Jin and H. Xiao, Recent advances in magnetic nanoparticles: Key applications, environmental insights, and future strategies, *Sustainable Mater. Technol.*, 2024, **40**, e00985.
121 P.-S. Wei, N. Thota, G. John, E. Chang, S. Lee, Y. Wang, Z. Ma, Y.-H. Tsai and K.-C. Mei, Enhancing RNA-lipid nanoparticle delivery: Organ- and cell-specificity and barcoding strategies, *J. Controlled Release*, 2024, **375**, 366–388.
122 N. Jain, A. Singh and D. Bhatia, DNA-amphiphilic nanostructures: synthesis, characterization and applications, *Nanoscale*, 2025, **17**(1), 18–52.
123 J. Schock Vaiani, M. Broekgaarden, J.-L. Coll, L. Sancey and B. Busser, In vivo vectorization and delivery systems for gene therapies and RNA-based therapeutics in oncology, *Nanoscale*, 2025, **17**(10), 5501–5525.
124 H. Yin, R. L. Kanasty, A. A. Eltoukhy, A. J. Vegas, J. R. Dorkin and D. G. Anderson, Non-viral vectors for gene-based therapy, *Nat. Rev. Genet.*, 2014, **15**(8), 541–555.
125 A. D. Judge, G. Bola, A. C. Lee and I. MacLachlan, Design of noninflammatory synthetic siRNA mediating potent gene silencing in vivo, *Mol. Ther.*, 2006, **13**(3), 494–505.
126 M. Wareing, A. N. Mhatre, R. Pettis, J. J. Han, T. Haut, M. H. Pfister, K. Hong, W. W. Zheng and A. K. Lalwani, Cationic liposome mediated transgene expression in the guinea pig cochlea, *Hear. Res.*, 1999, **128**(1–2), 61–69.
127 T. Okano, T. Nakagawa, T. Kita, T. Endo and J. Ito, Cell-gene delivery of brain-derived neurotrophic factor to the mouse inner ear, *Mol. Ther.*, 2006, **14**(6), 866–871.
128 X. Gao, Y. Tao, V. Lamas, M. Huang, W. H. Yeh, B. Pan, Y. J. Hu, J. H. Hu, D. B. Thompson, Y. Shu, Y. Li, H. Wang, S. Yang, Q. Xu, D. B. Polley, M. C. Liberman, W. J. Kong, J. R. Holt, Z. Y. Chen and D. R. Liu, Treatment of autosomal dominant hearing loss by in vivo delivery of genome editing agents, *Nature*, 2018, **553**(7687), 217–221.
129 H. Yin, R. L. Kanasty, A. A. Eltoukhy, A. J. Vegas, J. R. Dorkin and D. G. Anderson, Non-viral vectors for gene-based therapy, *Nat. Rev. Genet.*, 2014, **15**(8), 541–555.
130 J.-H. Lee, M. Y. Lee, Y. Lim, J. Knowles and H.-W. Kim, Auditory disorders and future therapies with delivery systems, *J. Tissue Eng.*, 2018, **9**, 2041731418808455.
131 G. Guerrini, D. Magrì, S. Gioria, D. Medaglini and L. Calzolai, Characterization of nanoparticles-based vaccines for COVID-19, *Nat. Nanotechnol.*, 2022, **17**(6), 570–576.
132 W. Tai, S. Feng, B. Chai, S. Lu, G. Zhao, D. Chen, W. Yu, L. Ren, H. Shi, J. Lu, Z. Cai, M. Pang, X. Tan, P. Wang, J. Lin, Q. Sun, X. Peng and G. Cheng, An mRNA-based T-cell-inducing antigen strengthens COVID-19 vaccine against SARS-CoV-2 variants, *Nat. Commun.*, 2023, **14**(1), 2962.
133 R. Verbeke, I. Lentacker, S. C. De Smedt and H. Dewitte, The dawn of mRNA vaccines: The COVID-19 case, *J. Controlled Release*, 2021, **333**, 511–520.
134 Y. Wu, S. Yu and I. de Lázaro, Advances in lipid nanoparticle mRNA therapeutics beyond COVID-19 vaccines, *Nanoscale*, 2024, **16**(14), 6820–6836.
135 S. S. Liu and R. Yang, Inner Ear Drug Delivery for Sensorineural Hearing Loss: Current Challenges and Opportunities, *Front. Neurosci.*, 2022, **16**, 867453.
136 J. Kim, Y. Eygeris, R. C. Ryals, A. Jozić and G. Sahay, Strategies for non-viral vectors targeting organs beyond the liver, *Nat. Nanotechnol.*, 2024, **19**(4), 428–447.
137 D. Yu, C. Sun, Z. Zheng, X. Wang, D. Chen, H. Wu, X. Wang and F. Shi, Inner ear delivery of dexamethasone using injectable silk-polyethylene glycol (PEG) hydrogel, *Int. J. Pharm.*, 2016, **503**(1), 229–237.
138 A. Erickson, P. A. Chiarelli, J. Huang, S. L. Levengood and M. Zhang, Electrospun nanofibers for 3-D cancer models, diagnostics, and therapy, *Nanoscale Horiz.*, 2022, **7**(11), 1279–1298.
139 B. Z. Chen, Z. Q. Zhao, M.-A. Shahbazi and X. D. Guo, Microneedle-based technology for cell therapy: current status and future directions, *Nanoscale Horiz.*, 2022, **7**(7), 715–728.
140 J. H. Lee, M. Y. Lee, Y. Lim, J. Knowles and H. W. Kim, Auditory disorders and future therapies with delivery systems, *J. Tissue Eng.*, 2018, **9**, 2041731418808455.
141 Y. Zhang, W. Zhang, A. H. Johnston, T. A. Newman, I. Pyykkö and J. Zou, Targeted delivery of Tet1 peptide functionalized polymersomes to the rat cochlear nerve, *Int. J. Nanomed.*, 2012, **7**, 1015–1022.
142 H. Zhou, X. Ma, Y. Liu, L. Dong, Y. Luo, G. Zhu, X. Qian, J. Chen, L. Lu, J. Wang and X. Gao, Linear polyethylenimine-plasmid DNA nanoparticles are ototoxic to the cultured sensory epithelium of neonatal mice, *Mol. Med. Rep.*, 2015, **11**(6), 4381–4388.
143 Y. Zhang, Z. Weikai, A. H. Johnston, T. A. Newman, P. Ilmari and J. Zou, Targeted delivery of Tet1 peptide functionalized polymersomes to the rat cochlear nerve, *Int. J. Nanomed.*, 2012, **7**, 1015–1022.
144 D. C. Gheorghe, A.-G. Niculescu, A. C. Bîrcă and A. M. Grumezescu, Nanoparticles for the Treatment of Inner Ear Infections, *Nanomaterials*, 2021, **11**(5), 1311.
145 M. G. Dindelegan, C. Blebea, M. Perde-Schrepler, A. D. Buzoianu and A. A. Maniu, Recent Advances and Future Research Directions for Hearing Loss Treatment Based on Nanoparticles, *J. Nanomater.*, 2022, **2022**(1), 7794384.
146 M. M. Goyal, N. J. Zhou, P. F. Y. Vincent, E. S. Hoffman, S. Goel, C. Wang and D. Q. Sun, Rationally Designed Magnetic Nanoparticles for Cochlear Drug Delivery: Synthesis, Characterization, and In Vitro Biocompatibility in a Murine Model, *Otol. Neurotol. Open*, 2022, **2**(3), e013.
147 J. Zou, R. Sood, S. Ranjan, D. Poe, U. A. Ramadan, P. K. Kinnunen and I. Pyykkö, Manufacturing and in vivo inner ear visualization of MRI traceable liposome nanoparticles encapsulating gadolinium, *J. Nanobiotechnol.*, 2010, **8**, 32.

148 A. S. Arbab, W. Liu and J. A. Frank, Cellular magnetic resonance imaging: current status and future prospects, *Expert Rev. Med. Devices*, 2006, **3**(4), 427–439.
149 X. Wu, D. Ding, H. Jiang, X. Xing, S. Huang, H. Liu, Z. Chen and H. Sun, Transfection using hydroxyapatite nanoparticles in the inner ear via an intact round window membrane in chinchilla, *J. Nanopart. Res.*, 2012, **14**(1), 708.
150 M. Bartneck, H. A. Keul, M. Wambach, J. Bornemann, U. Gbureck, N. Chatain, S. Neuss, F. Tacke, J. Groll and G. Zwadlo-Klarwasser, Effects of nanoparticle surface-coupled peptides, functional endgroups, and charge on intracellular distribution and functionality of human primary reticuloendothelial cells, *Nanomedicine*, 2012, **8**(8), 1282–1292.
151 W. Zhang, Y. Zhang, R. Sood, S. Ranjan, E. Surovtseva, A. Ahmad, P. K. Kinnunen, I. Pyykkö and J. Zou, Visualization of intracellular trafficking of Math1 protein in different cell types with a newly-constructed nonviral gene delivery plasmid, *J. Gene Med.*, 2011, **13**(2), 134–144.
152 I. Pyykkö, J. Zou, W. Zhang and Y. Zhang, Nanoparticle-based delivery for the treatment of inner ear disorders, *Curr. Opin. Otolaryngol. Head Neck Surg.*, 2011, **19**(5), 388–396.
153 W. Zhang, Y. Zhang, R. Sood, S. Ranjan, E. Surovtseva, A. Ahmad, P. K. J. Kinnunen, I. Pyykkö and J. Zou, Visualization of intracellular trafficking of Math1 protein in different cell types with a newly-constructed nonviral gene delivery plasmid, *J. Gene Med.*, 2011, **13**(2), 134–144.
154 T. Sakamoto, T. Nakagawa, R. T. Horie, H. Hiraumi, N. Yamamoto, Y. S. Kikkawa and J. Ito, Inner ear drug delivery system from the clinical point of view, *Acta Otolaryngol. Suppl.*, 2010, **563**, 101–104.
155 R. T. Horie, T. Sakamoto, T. Nakagawa, Y. Tabata, N. Okamura, N. Tomiyama, M. Tachibana and J. Ito, Sustained delivery of lidocaine into the cochlea using poly lactic/glycolic acid microparticles, *Laryngoscope*, 2010, **120**(2), 377–383.
156 C. Sun, X. Wang, Z. Zheng, D. Chen, X. Wang, F. Shi, D. Yu and H. Wu, A single dose of dexamethasone encapsulated in polyethylene glycol-coated polylactic acid nanoparticles attenuates cisplatin-induced hearing loss following round window membrane administration, *Int. J. Nanomed.*, 2015, **10**, 3567–3579.
157 T. Nakagawa, T. Sakamoto, H. Hiraumi, Y. S. Kikkawa, N. Yamamoto, K. Hamaguchi, K. Ono, M. Yamamoto, Y. Tabata, S. Teramukai, S. Tanaka, H. Tada, R. Onodera, A. Yonezawa, K. Inui and J. Ito, Topical insulin-like growth factor 1 treatment using gelatin hydrogels for glucocorticoid-resistant sudden sensorineural hearing loss: a prospective clinical trial, *BMC Med.*, 2010, **8**, 76.
158 T. Sakamoto, N. Takayuki, R. T. Horie, H. Harukazu, Y. Norio, Y. S. Kikkawa and J. Ito, Inner ear drug delivery system from the clinical point of view, *Acta Otolaryngol. Suppl.*, 2010, **130**(563), 101–104.
159 R. T. Horie, T. Sakamoto, T. Nakagawa, Y. Tabata, N. Okamura, N. Tomiyama, M. Tachibana and J. Ito, Sustained delivery of lidocaine into the cochlea using poly lactic/glycolic acid microparticles, *Laryngoscope*, 2010, **120**(2), 377–383.
160 M. E. Martin and K. G. Rice, Peptide-guided gene delivery, *AAPS J.*, 2007, **9**(1), E18–E29.
161 J. C. Leclère, R. Marianowski and T. Montier, Gene therapy for hearing loss: Current status and future prospects of non-viral vector delivery systems, *Hear. Res.*, 2024, **453**, 109130.
162 Z. Wu, H. Yang and P. Colosi, Effect of Genome Size on AAV Vector Packaging, *Mol. Ther.*, 2010, **18**(1), 80–86.
163 H. C. J. Ertl, Immunogenicity and toxicity of AAV gene therapy, *Front. Immunol.*, 2022, **13**, 975803.
164 Z. Yuan, B. Li, W. Gu, S. Luozhong, R. Li and S. Jiang, Mitigating the Immunogenicity of AAV-Mediated Gene Therapy with an Immunosuppressive Phosphoserine-Containing Zwitterionic Peptide, *J. Am. Chem. Soc.*, 2022, **144**(44), 20507–20513.
165 Z. Zhao, Z. Han, K. Naveena, G. Lei, S. Qiu, X. Li, T. Li, X. Shi, W. Zhuang, Y. Li, Y. Qiao and H. Liu, ROS-Responsive Nanoparticle as a Berberine Carrier for OHC-Targeted Therapy of Noise-Induced Hearing Loss, *ACS Appl. Mater. Interfaces*, 2021, **13**(6), 7102–7114.
166 J. G. Naples, L. E. Miller, A. Ramsey and D. Li, Cochlear protein biomarkers as potential sites for targeted inner ear drug delivery, *Drug Delivery Transl. Res.*, 2020, **10**(2), 368–379.
167 L. P. Rybak, C. A. Whitworth, D. Mukherjea and V. Ramkumar, Mechanisms of cisplatin-induced ototoxicity and prevention, *Hear. Res.*, 2007, **226**(1), 157–167.
168 S. Roy, A. H. Johnston, T. A. Newman, R. Glueckert, J. Dudas, M. Bitsche, E. Corbacella, G. Rieger, A. Martini and 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.*, 2010, **390**(2), 214–224.
169 Y. Tao, X. Liu, L. Yang, C. Chu, F. Tan, Z. Yu, J. Ke, X. Li, X. Zheng, X. Zhao, J. Qi, C.-P. Lin, R. Chai, G. Zhong and H. Wu, AAV-ie-K558R mediated cochlear gene therapy and hair cell regeneration, *Signal Transduction Targeted Ther.*, 2022, **7**(1), 109.
170 J. Wang, J. Zheng, H. Wang, H. He, S. Li, Y. Zhang, Y. Wang, X. Xu and S. Wang, Gene therapy: an emerging therapy for hair cells regeneration in the cochlea, *Front. Neurosci.*, 2023, **17**, 1177791.
171 H. Sun, A. Huang, H. Liu and S. Cao, Gene Therapy for the Inner Ear: Progress and Prospects, in *Emerging Trends in Cell and Gene Therapy*, ed. M. K. Danquah and R. I. Mahato, Humana Press, Totowa, NJ, 2013, pp. 595–623.
172 T. Wei, Q. Cheng, L. Farbiak, D. G. Anderson, R. Langer and D. J. Siegwart, Delivery of Tissue-Targeted Scalpels: Opportunities and Challenges for In Vivo CRISPR/Cas-Based Genome Editing, *ACS Nano*, 2020, **14**(8), 9243–9262.
173 L. Wang, R. Zhang, L. Jiang, S. Gao, J. Wu and Y. J. B. S. Jiao, Biomaterials as New Option for Treating Sensorineural Hearing Loss, *Biomater. Sci.*, 2024, **12**(16), 4006–4023.
174 T. Okano, T. Nakagawa, T. Kita, T. Endo and J. Ito, Cell-Gene Delivery of Brain-Derived Neurotrophic Factor to the Mouse Inner Ear, *Mol. Ther.*, 2006, **14**(6), 866–871.

175 I. Pyykkö, J. Zou, A. Schrott-Fischer, R. Glueckert and P. J. A. Kinnunen, An overview of nanoparticle based delivery for treatment of inner ear disorders, *Methods Mol. Biol.*, 2016, **1427**, 363–415.
176 J. Qi, F. Tan, L. Zhang, L. Lu, S. Zhang, Y. Zhai, Y. Lu, X. Qian, W. Dong, Y. Zhou, Z. Zhang, X. Yang, L. Jiang, C. Yu, J. Liu, T. Chen, L. Wu, C. Tan, S. Sun, H. Song, Y. Shu, L. Xu, X. Gao, H. Li and R. Chai, AAV-Mediated Gene Therapy Restores Hearing in Patients with DFNB9 Deafness, *Adv. Sci.*, 2024, **11**(11), e2306788.
177 J. Lv, H. Wang, X. Cheng, Y. Chen, D. Wang, L. Zhang, Q. Cao, H. Tang, S. Hu, K. Gao, M. Xun, J. Wang, Z. Wang, B. Zhu, C. Cui, Z. Gao, L. Guo, S. Yu, L. Jiang, Y. Yin, J. Zhang, B. Chen, W. Wang, R. Chai, Z. Y. Chen, H. Li and Y. Shu, AAV1-hOTOF gene therapy for autosomal recessive deafness 9: a single-arm trial, *Lancet*, 2024, **403**(10441), 2317–2325.
178 E. S. Ho, *Local Delivery of PACAP Attenuates Inflammation and Promotes Functional Recovery in a Mouse Model of Stroke Injury*, University of Toronto (Canada), Canada – Ontario, CA, 2023, p. 199.
179 M. Izumikawa, R. Minoda, K. Kawamoto, K. A. Abrashkin, D. L. Swiderski, D. F. Dolan, D. E. Brough and Y. Raphael, Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals, *Nat. Med.*, 2005, **11**(3), 271–276.