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Nanomedicine: Nanotechnology, Biology, and Medicine
46 (2022) 102599

nanomedicine
Nanotechnology, Biology, and Medicine

nanomedjournal.com

Potential nanotechnology-based diagnostic and therapeutic approaches for Meniere's disease

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Revised 19 August 2022

Abstract

Meniere's disease (MD) is a progressive inner ear disorder involving recurrent and prolonged episodes or attacks of vertigo with associated symptoms, resulting in a significantly reduced quality of life for sufferers. In most cases, MD starts in one ear; however, in one-third of patients, the disorder progresses to the other ear. Unfortunately, the etiology of the disease is unknown, making the development of effective treatments difficult. Nanomaterials, including nanoparticles (NPs) and nanocarriers, offer an array of novel diagnostic and therapeutic applications related to MD. NPs have specific features such as biocompatibility, biochemical stability, targetability, and enhanced visualization using imaging tools. This paper provides a comprehensive and critical review of recent advancements in nanotechnology-based diagnostic and therapeutic approaches for MD. Furthermore, the crucial challenges adversely affecting the use of nanoparticles to treat middle ear disorders are investigated. Finally, this paper provides recommendations and future directions for improving the performances of nanomaterials on theragnostic applications of MD.

Keywords: Meniere's disease; Nanotechnology; Nanoparticles; Theragnostic applications; Nano-based hybrid systems

Introduction

Menieres disease (MD) is a debilitating condition of the inner ear characterised by the hallmark balance and hearing symptoms, spontaneous vertigo, fluctuating hearing loss, tinnitus, and aural fullness.[1] However, as these indications do not always present themselves equally at the beginning of the disease, and largely worsen over time, MD is regarded as a progressive disorder.[2] Discussions about the symptoms and treatment of MD are provided elsewhere.[3] Overall, MD symptoms are unpleasant and result in a reduced quality of life. Often, patients may undergo a complete labyrinthectomy of the ear as a treatment from their debilitating symptoms. Epidemiological studies demonstrate that MD afflicts approximately 0.02 % to 0.5 % of the population, which varies among nations and studies[4,5], resulting in a multi-million dollar socio-economic burden in many countries.[6]

A histopathological hallmark of the disease is Endolymphatic Hydrops (EH) - defined as the overaccumulation of inner ear fluid (endolymph), within the membranous labyrinth. Although the cause of EH in MD is unknown, it is thought to involve multiple aetiological factors, such as genetics, infection (viral or bacterial), autoimmune disorder, metabolic injury, and allergy, among others.[7–9] One possible cause of EH is via endolymph malabsorption, where the endolymphatic duct limits endolymph flow to the sac. Here is it possible that MD could be caused by congenital narrowing, acquired scarring or fibrosis of the endolymphatic duct, or by blocking the endolymphatic sac by a viral infection, allergy mediated, cellular debris, or immune complexes. Here, the sac may respond by secreting glycoproteins, producing an excess of endolymph as a consequence. This may be analogous to the event which produces an acute MD attack or vertiginous event. Unfortunately, it is still unclear how these

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https://doi.org/10.1016/j.nano.2022.102599
1549-9634/

factors relate to MD pathophysiology and cause acute attacks. Although the cause of EH in MD is unknown, it is thought to involve multiple aetiological factors, such as genetics, infection, autoimmunity, and allergy.[7–9] Unfortunately, it is still unclear how these factors relate to MD pathophysiology and cause acute attacks. Discussion about symptoms and treatment of MD are provided elsewhere.[3]

In order to explain the cause of severe MD attacks, several theories have been proposed, such as the rupture theory, hydrostatic pressure theory[10], ischemic theory, and inflammatory theory. However, many of these theories conflict with clinical and experimental observations, and for this reason, more comprehensive experimental investigations into the cause of acute attacks in MD are needed. A more thorough discussion regarding the underlying mechanism of hydrops related to MD is provided in past proposals.[11]

The ability to diagnose and treat MD continues to be challenging, stemming in part from anatomical barriers to the inner ear, where it is encased behind the dense temporal bone in the skull.[12] Because of this, it is difficult to record from the sensory receptors of the cochlea and the balance organs and to treat them with pharmacological agents and other therapeutics.[13] Therefore, novel tools are needed with improved access to the inner ear for diagnostics and therapy of MD.

The emergence of nanotechnology and nanomaterials has recently improved conventional diagnostic and therapeutic approaches[14–16] and found numerous engineering applications.[17–20] Nanoscale materials are defined by their submicron size and high surface aspect ratio. Nanoparticles (NPs) are inorganic molecules whose sizes vary from 1 to 1000 nm and have various specific properties, making them desirable in treating diseases.[16,21–23] They can generally be affected by external forces such as ultrasound, heat, magnetism, and light, and also conjugated with various labels, all of which make them good candidates for drug delivery, therapy and imaging.[24–26] Moreover, they are capable of crossing biological systems (such as, single cells, tissues, or organs) compared with other larger organic materials.[27] There are numerous types of Nanoparticles which can be used to treat inner ear dysfunction. NPs can pass through the round window membrane (RWM) or oval window (OW). NP delivery via the OW was first introduced in a study by Saijo et al.[28] as a secondary route for transferring a horseradish peroxidase (HRP) tracer from the middle ear cavity to the inner ear. The OW pathway can provide a more permeable route for specific drug formulations and offer decent accessibility to the vestibular system, which has merit for treating balance dysfunction. RWM delivery is regarded as a safe and effective way of pharacologically targeting cells in the cochlea.[29] Various research has revealed that the efficiency of these two pathways varies depending on the types of particles. For instance, in a study by Zou et al.[30], the RWM demonstrated more efficiency for the penetration of a specific NP. Other studies demonstrated that OW was a more permeable route for other certain substances.[31–33]

NPs have proven to be potent tools to overcome the anatomical and physiological barriers of the ear, for various purposes, including targeted drug release, protecting pharmacological formulations up to the desired site, facilitating transmembrane transport, increasing cell uptake, and reducing required doses and side effects.[34,35] NPs have been recently used in therapeutic strategies to treat inner ear infections due to their interesting characteristics and biological and chemical properties.[36] Nanocarriers have notably been used to carry biomolecules for inner ear disease therapy.[37] In recent years, numerous sorts of NPs, such as inorganic nanoparticles, soft material nanoparticles, nanosized polymers, peptides, silicas, and metal-organic frameworks (MOFs), were successfully used as drug delivery approaches for a variety of inner ear treatments.[38] The size of most used NPs in inner ear treatment and diagnosis is lower than 200 nm.[39] In this study we categorised NPs based on their functionality in inner ear diagnostic or treatment applications, as illustrated in Fig. 1. Metallic NPs have been used for inner ear imaging purposes mentioned below. NPs that have been already utilized or have the potential to be used as nanotechnology-based drug delivery systems are also mentioned below, where the nanocarriers are classified into nanogels, polymeric NPs, inorganic NPs and Lipid NPs.

This paper aims to thoroughly discuss the recently developed approaches in the field of nanotechnology on the diagnosis and theragnostic application of MD. Moreover, some of the technologies in nanotechnology discussed here have not been directly used in MD yet, but they have great potential to help patients with this disease in the future. The application of nanotechnology in MD in various categories like diagnosis, drug delivery, treatment, and clinical study has been critically discussed.

Application of Nanoparticles for Inner Ear Imaging

Diagnosis of MD with various technology, such as Magnetic Resonance Imaging (MRI), has been a topic of debate due to potential issues with signal-to-noise ratios and the difficulty in delineating clear biological boundaries within the inner ear.[40,41] Despite this, these modalities have merit in non-invasively determining the location, severity and progression of EH, as a proxy for MD severity. Despite the challenges, several findings have shown the positive use of MRI and Computed Tomography (CT) in identifying EH for the diagnosis of MD[42], which are discussed in this section.

Magnetic Resonance Imaging

MRI has been widely employed as a diagnostic approach to visualise EH in both the cochlea and vestibular system.[41–44] EH visualization might be classified based on either a semi-quantitative ratio between endolymph and perilymph liquids, or the difference between the saccular and utricular structures.[45] In 2016, a qualitative but rapid assessment of EH was reported by Ito et al.[46] where the endolymphatic space (ELS) in patients with MD was compared with that in healthy controls using 3-T MRI at 4 h after intravenous administration of Gadolinium (Gd). It was demonstrated that the incident ratios of cochlear and/or vestibular EH in the affected MD ears were considerably higher than in controls. A recent study conducted by the same group also explored the quantitative and precise evaluation of EH in MD patients by MRI.[47] In that research, EH was characterised by

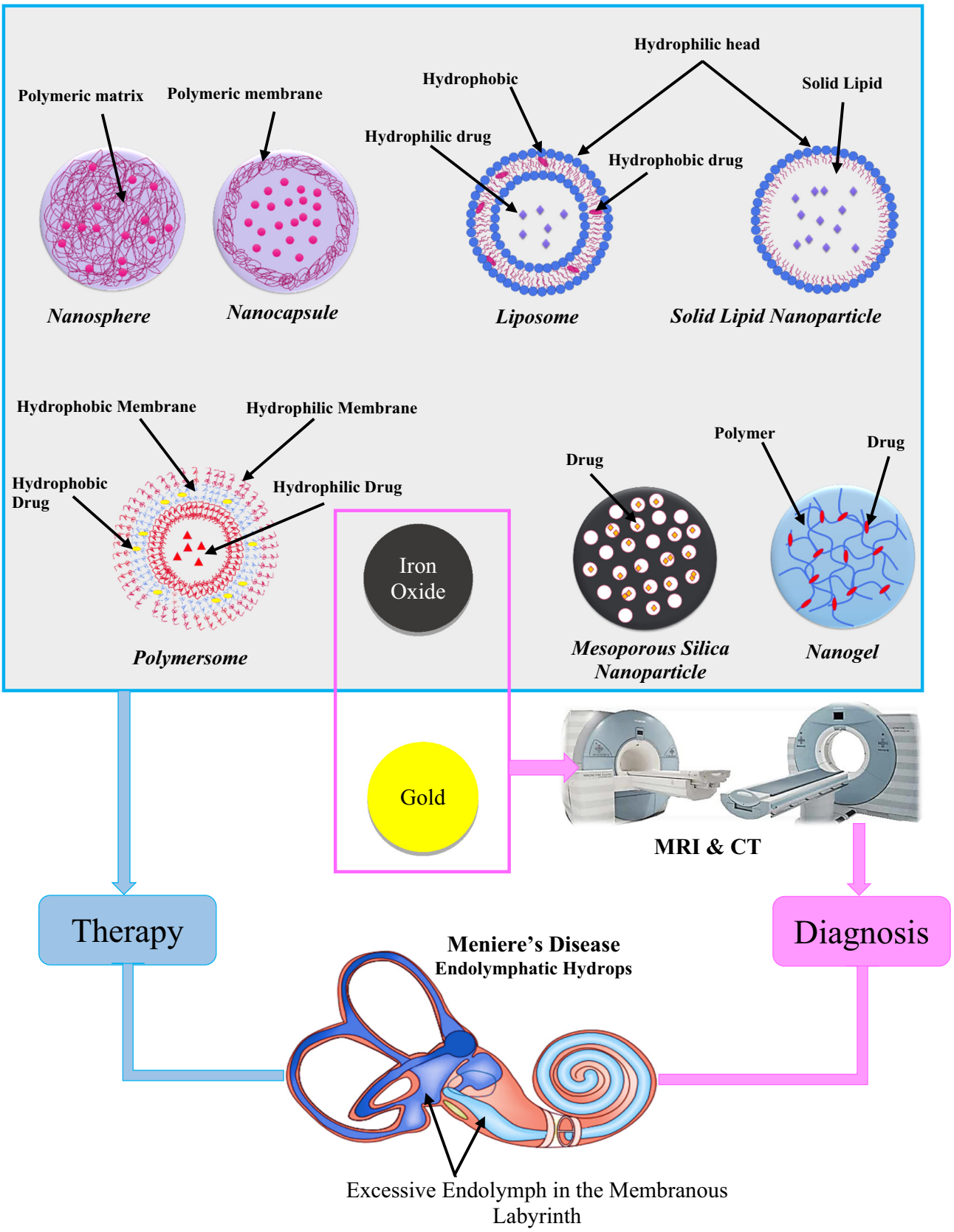

Fig. 1. Schematic representation of promising nano-based materials used for the potential diagnosis and therapy of MD and other inner ear diseases.

volumetric measurements of the endolymphatic space (ELS) and its comparison in bilateral/unilateral MD (bMD/uMD) patients and healthy control groups. The three-dimensional (3D) analysis of EH with MRI revealed that the ELS percentages (the volume percentage of the ELS to that of the total fluid space) in the inner ear, cochlea, vestibule, and semicircular canals, were significantly higher in the affected ear of persons with bMD/uMD than control groups.[47] Similar research was performed in another study[48] for quantitative assessment of EH by volumetric MRI. Although the sample size, patients characteristics, contrast agents and applied methods were different in those researches, their results were inconsistent. In addition, MRI has been widely employed to explore whether cochleovestibular nerves display imaging signs of axonal loss.[45] Existing endolymphatic hydrops strategies developed based on MRI are explained in.[49]

In most of the previous studies, Gd compounds are employed as beneficial agents to enhance contrast in MRI inner ear images[50] because they are hydrophilic, thermodynamically stable, and kinetically inert chelates.[51] Based on a study conducted in 2018,[52] MRI with intratympanic (IT) Gd administration can be deemed as a standard diagnostic process for MD. It is demonstrated that MRI can potentially lead to improved r-results than other audio-vestibular tests, like the caloric test, video head impulse test (vHIT), and cervical-vestibular-evoked-myogenic-potentials (cVEMP) in the diagnosis of suspected MD.[52] However, the use of these contrast agents faced several challenges.[51] It is revealed that some fraction of the injected Gd is retained in both human and animal bodies, which raises serious concerns about the long-term toxicological properties of these contrast agents.[51,53] Therefore, there has been a need for alternative contrast agents. Novel NPs based on transition metal oxides can be considered attractive contrast agents because of their biocompatibility and low toxicity.[54,55]

Metallic NPs, including metals or iron oxide, have garnered much attention in translational applications related to biomedical use because of their biocompatible features.[56] However, they are non-biodegradable and cannot be eliminated by the body.[57] Biodegradable polymers such as polylactic acid (PLA), poly-e-caprolactone (PCL), poly lactic-co-glycolic acid (PLGA) and chitosan, can be utilized to convert metal NPs into biodegradable ones.[58,59] Therefore, NPs can be naturally degraded under biological conditions. Among different metallic NPs, the toxicity of SPIONs is lower than others.[60] Moreover, the cytotoxicity of gold nanoparticles, which can be considered as a potential contrast agent for MD diagnostic approaches, is low.[61]

Superparamagnetic iron oxide nanoparticles (SPIONs) have recently been utilized as contrast agents in molecular and cell imaging in order to distinguish diseased tissues from healthy ones.[62,63] In particular, SPIONs can create dark contrast against bright signals within the fluids by suppressing the signals in T2-weighted MRI. Thus, they can be deemed potent contrast agents.[64] Moreover, Zou et al.[65] studied the functionality of SPIONs by presenting a novel NP pluronic VR F127 copolymer overlaying oleic acid-coated SPIONs (POA@SPIONs), which has demonstrated exceptional T2 contrast enhancement. Inner ear administration of POA@SPIONs allowed visualization of the endolymphatic compartment due to an enhanced T2 signal relative to the perilymph. This feature assists in investigating the health of perilymph-endolymph barriers, which might be distorted as a result of endolymphatic hydrops in MD.[66]

Superparamagnetic maghemite nanoparticles, abbreviated as CAN-γ-Fe_2O_3 NPs, are other derivatives of SPIONs, which are comprised of Fe_3O_4 NPs via ceric ammonium nitrate (CAN) oxidation. These NPs can enter via the round and oval windows, providing good penetration to the inner ear, with a strong T2 contrast effect for imaging.[33] Moreover, they can potentially be employed in molecular imaging. Importantly, within a strong magnetic field, such as a 7 T MRI, CAN-γ-Fe2O3 NPs demonstrated great stability.[33] They also have a cationic surface charge which may facilitate enhanced delivery into the oval and round windows of the cochlea, in comparison with POA@SPIONs.[67]

There are some clinically approved nanoparticles, such as sulfur colloid, albumin colloid, stannous fluoride (SnF2) colloid, and dextran-coated iron oxide (ferumoxtran), which are used for radionuclide imaging and MRI.[68] We focus on MRI contrast agents as MRI is has utility in MD diagnosis. Among various iron oxide nanoparticles, Feraheme®; Combidex® and Sinerem® have been approved by the U.S. Food and Drug Administration (FDA) as magnetic resonance imaging agents for clinical use.[69] Other SPION-based contrast agents, including Feridex and Clariscan, have received FDA approval, however, their development was halted due to poor efficiency compared to conventional contrast agents.[70] Among all, Feraheme® (ferumoxytol) has shown great potential in biomedical applications, including MRI, and drug delivery. Ferumoxytol is an ultrasmall SPION that can result in image contrast for MRI via T1, T2, and T2* shortening. The coated version of this NP can remain in blood circulation for a long time.[71] However, the long-term deposition of this NP in the human brain has not been investigated yet.

Computed Tomography (CT)

One of the main challenges in finding the etiology of MD, and EH, is the isolated human inner ear surrounded by hard bone. Using computed tomography (CT) to monitor the inner ear can be beneficial to to extract structural information regarding EH and MD.[72] For example, employing high-resolution computed tomography in a case-control study revealed that some anatomical variations, like the individualization of lower rates of the vestibular aqueduct, might be more common in the affected ears of MD sufferers.[73] Moreover, different angular trajectories of the vestibular aqueducts have been observed in MD patients with a degenerative and hypoplastic endolymphatic sac (ES) by CT-imaging-based tools.[74,75] However, CT imaging tools suffer from limited resolution of soft tissues and contrast agents. Applying conventional contrast agents such as organic iodinated molecules to enhance image qualities has faced several major challenges.[76] Firstly, their small molecular weight results in short circulation time, as rapidly metabolized by kidneys, which can result in acute kidney disease.[76] Secondly, their low biocompatible characteristics led to iterating side effects such as vomiting, high fever and so forth. Thirdly, due to their inability to specifically target tissues, a higher volume of these contrast agents is required, which can be harmful to patients with allergic reactions or kidney complications.

Development of NPs with interesting characteristics, such as excellent biocompatibility, prolonged circulation time and ability in targeting, could resolve many mentioned issues of the conventional contrast agents.[76,77] Micro CT visualization of silver NPs (AgNPs) revealed that these NPs can provide access to the inner ear through the round and oval windows in a dose-dependent manner.[78] Nevertheless, it is reported that the minimum concentration of AgNPs for detection by micro-CT is 37 mM, which makes it unsuitable for clinical application because of its potential toxic effects.[78] Targeted gold nanoparticles (AuNPs) are another group of contrast agents that can be used for inner ear diagnostic imaging, for example, in micro-CT.[79] Among various metal NPs as contrast agents (Ag, Au, Cu, Hg, and Pt), AuNPs are regarded as the most stable ones, which as of now are extensively used in biomedical fields.[80–82] For instance, a type of AuNPs known as gold nanoclusters (AuNCs) has exhibited appropriate stability compared to other molecular dyes used in fluorescence imaging.[83,84] Although the presence of AuNPs have led to a very limited enhancement of CT images, there is no clear evidence of AuNP toxicity in comparison to iodine contrast agents, which are toxic to some patients.[63,79] Another study revealed that liposomal iodine NPs have the ability to produce improved CT image quality of the labyrinth. They have several merits over gold-based contrast agents, including reduced cost, FDA approval, and broad availability.[63]

Other contrast agents, such as Bismuth sulfide NPs (BiNPs), have been used for the visualization of the tumor boundaries and may have the potential to be used in inner ear imaging based on previous findings[54]. These NPs can be cleared from the body at a slower pace compared to the iodine agents[85] and absorb X-ray energy five times more.[54] However, following the application of bismuth salt, there were high levels of cytotoxicity, therefore coated bismuth nanoparticles are developed to counteract this. Further, it has been shown that coating BiNPs with dopamine and polyethylene glycol (PEG) did not result in cytotoxicity at the concentration of 1 mg Bi/mL.[85]

Given the problems with molecular imaging of the labyrinth, one of the most promising solutions is a standard CT scan utilising optical imaging, which uses fluorescent targeted contrast agents to identify both targeted features of the inner ear. Here, enhancements in imaging technology combined with the implementation of targeted contrast agents can make it possible to detect molecular and morphological changing within the inner ear in a safe and targeted way. Due to favorable properties and characteristics, NPs can be employed as imaging contrast agents or drug delivery systems in MD, as listed in Table 1. Although extremely large-sized particles have been tested in the reports, the international consensus is that the size of nanoparticles should be limited to below 200 nm.[39]

Nanotechnology for Treatment and Drug Delivery in Meniere's Disease

The development of new drug delivery systems with minimal side effects is important as a treatment for MD to relieve debilitating symptoms. Several conservative strategies also have been developed to manage the severity of MD symptoms such as dietary restrictions.[109,110] Other more crude and ablative treatment options include nerve section and labyrinthectomy, where the sensory nerves or end organs are surgically ablated to halt debilitating vertiginous symptoms. However, invasive and irreversible procedures[111,112] are unpleasant for most patients, with additional consequences such as inflammation in the middle ear cavity following surgery.[113] espite recent progress, one of the biggest hurdles of pharmacological treatment in otolaryngology remains to be the safe and efficacious delivery of compounds into the labyrinth.[2] That is the combined toxicological and pharmacokinetic problems.

In order to treat inner ear disorders, like MD, generally, two types of drug administrations are available: *systematic* and *local drug administration. Systematic drug administration* is a relatively convenient method to ensure pharmacological agents make their way to the inner ear via the bloodstream;[114] however, this may lead to undesirable side effects, such as sub-therapeutic concentration of the drug at the desired tissue and non-selective drug-receptor binding at other sites, with the potential for a range of side effects By contrast, *Local drug delivery* faces several complications due to the anatomy and physiology of the inner ear.[12] The slow circulation of perilymph in the inner ear and the blood-labyrinth barrier (BLB) hinder drug delivery into the fatty tissues and cells of the cochlear and vestibular system. In addition, the round and oval window within the inner ear play an important role in preventing the permeability of large particles into the cochlea.[35] Furthermore, the stability of drug concentration in the cochlea is mostly low, which reduces pharmacological effectiveness.

In terms of local drug delivery, intratympanic (IT) injection and direct microinjection have demonstrated effectiveness. IT injection of drugs, such as gentamicin or dexamethasone (DEX), is commonly used as a therapeutic solution that assists with controlling vertigo in patients suffering from MD.[116–118] Although this method is widely used in treatments of MD, its efficacy is restricted due to the existence of the eustachian tube; where a large proportion of drugs are cleared via this mechanism.[119] Another drug delivery approach is called direct microinjection, which improves a lack of control linked to IT injections, which may increase the risk of inner ear damage or trauma.[120] Despite the use of techniques, several limitations exist associated with pharmacokinetics, and hence improved methods would improve drugs delivery for treatment of the inner ear.[121,122] Emergence of NPs and their special properties in delivering various types of therapeutics has addressed many challenges of conventional drug delivery systems.[123] By creating a protective layer around the molecules, NPs can obviate challenges related to drug degradation. Furthermore, their capability to tune physiochemical properties of the surface, such as charge and hydrophilicity of particles, results in a prolonged half-life during circulation time, as well as more tissue penetration and more cellular uptake. Nanoparticle-based drug delivery methods can also release a sustained dosage of drugs and provide cell-specific delivery. As a result, they have the potential to be employed as novel carriers for drug delivery.[123] A summary of the recent advances in nano-based treatment and drug delivery strategies can be found in Table 2.

Nano-based Targeted Drug Delivery Systems for Meniere's Disease

Drug delivery is an important tool to treat inner ear diseases. Viral vectors offer a potential treatment strategy, and recent studies

Table 1
A list of NPs capable of being used for diagnostics and treatment in MD.

Name	Size range (nm)	Properties	Diagnostic and therapeutic applications for MD	Application in other Inner ear disorders	FDA approval	References
Superparamagnetic iron oxide nanoparticles (SPIONs)	100, 200, and 500	Non-toxic; Biocompatible; Biodegradable; Control by an external magnetic field; Tendency to aggregation	MRI Dexamethasone (DEX)	Sudden sensorineural hearing loss, MD, Noise-induced hearing loss	Yes	86–91
Gold nanoparticles (AuNPs)	<10	Proper biocompatibility; Inducing no damage to the blood-brain barrier; Various surface modifications	CT scan	Sensorineural hearing loss,	No	39,79
Nanogels	45–250	High levels of water content; Might be sensitive to temperature	Dexamethasone and gentamicin	Sensorineural hearing loss	No	21,34,92,93
PLGA NPs	100–1000	Transport hydrophilic/hydrophobic agents; Stable; Biodegradable; Tiny alterations in size lead to changes in delivery efficacy	Dexamethasone	Noise-induced hearing damage, Sudden sensorineural hearing loss	Yes	94–96
Polymersomes	40–200	Drug stabilization; Sustained drug release	Dexamethasone	Sensorineural hearing loss, cochlear synaptopathy (Noise-induced hearing loss)	No for use in the ear	2,97–99
Mesoporous Silica nanoparticles	50–200	Degradable; Proper biocompatibility; Easy functionalisation; Unknown transport rate into middle ear barriers	Gentamicin	Noise-induced hearing loss,	No	21,100–102
Lipid nanoparticles	30–200	Easy fabrication; Controlled release; Transport hydrophilic/hydrophobic agents	Dexamethasone	Sudden sensorineural hearing loss, Noise-induced hearing loss, Acute Acoustic Stress-Induced Cochlea Damage	No	21,39,103–106
Liposomes	80–200	Low levels of toxicity; Straightforward preparation and commercialization	CT scan Dexamethasone	Sudden sensorineural hearing loss, Noise-induced hearing loss	Yes	21,39,63,93,97,107,108

using viral vectors for drug delivery have displayed low toxicity. However, potential hazards remain with this technique in a clinical application, especially given the delicacy of inner ear structures and their locality to other vulnerable tissues, like the brain.[136] More discussion on challenges associated with drug delivery in the inner ear is provided in the following references.[137,138] Employing NPs in medicine has addressed several issues of conventional drug administration.[39,139,140] NPs not only can improve the systematic delivery of drugs from the bloodstream into various labyrinthine structures, but they can also assist in local drug administration into the inner ear. Notably, NPs can encapsulate medicine, make drug structures more sustainable, increase drug uptake, boost drug diffusion through biological membranes, such as the RWM, and facilitate drug passage to the cochlea.[36,141] The development of smart nanocarriers for the targeted delivery of multiple therapeutic agents may future benefit within the field.[138,142]

Nanocarriers, also known as nanoscale drug delivery systems, can take compounds into the inner ear in a sustained way and compensate for some drug properties, including degradation, poor solubility, low half-life, and limited access to biological membranes.[34,39,137,143,144] Size, material, shape, surface charge, and biodegradability properties have made nanocarriers suitable for various applications. Moreover, based on their properties, NPs are able to pass the middle ear barriers rapidly and reach the

Table 2
Recent developments in nano-based therapeutics and drug delivery methods.

Reference	Study model	Administration method	Strategies	Benefits/shortcomings
Dai et al.[92]	In vivo (Guinea pigs)	Intratympanic injection	Chitosan/glycerophosphate (CS/GP)-based thermosensitive hydrogel encapsulate PLGA NPs containing α-2 b	*Benefits:* Allowed longer delivery of drugs to the inner ear (1.5–3-fold). *Limitations:* No information about the pharmacodynamics (exact distribution and elimination).
Cai et al.[124]	In vivo (Guinea pigs)	Intratympanic	*Salvia miltiorrhiza* and *Panax notoginseng* encapsulated in PLGA NPs	*Benefits:* 1) Powerful ability in transporting single or compound drugs into the perilymph via the round window membrane (RWM) 2) Improved inner ear bioavailability by encapsulation. *Limitations:* Drug delivery regimen in vivo differs to clinical administration.
Sun et al.[94]	In vivo (Guinea pigs)	Intraperitoneal	polyethyleneglycol-coated polylactic acid (PEG-PLA) nanoparticles loaded with dexamethasone	*Benefits:* Enhanced the protective efficacy of dexamethasone versus cisplatin induced hearing loss (CIHL) after systemic application. *Limitations:* Investigation is done in a single, large dose model, while in the clinic a multiple dose model is used.
Sun et al.[125]	In vivo (Guinea pigs)	Intracochlear	PEG-PLA stealth nanoparticles loaded with dexamethasone	*Benefits:* Greater protective efficacy for dexamethasone vs. CIHL via local RWM diffusion because of prolonged delivery. Limitation: Drug delivery regimen in vivo differs to clinical administration.
Kuang et al.[126]	Zebrafish embryos	Intracochlear	PLGA nanoparticles conjugated with SS-31 peptide	*Benefits:* Mitochondrial specific accumulation in cochlear hair cells to reduce auditory receptor damage associated with aminoglycoside antibiotics administration such as gentamicin. *Limitations:* Non-mammalian model. Limited translation to clinic.
Ding et al.[32]	In vivo (Guinea pigs)	Intratympanic	Distribution of CS NPs in cochlear and vestibular organs	*Benefits:* The oval window route simplifies the transport of CS NPs into inner ear. *Limitations:* inner ear delivery attributed to

(continued on next page)

Table 2 (*continued*)

Reference	Study model	Administration method	Strategies	Benefits/shortcomings
				diffusion across the RWM and oval window is unclear
Surovtseva et al.[127]	In vivo (Rat)	Intracochlear	Conjugating A665 and A666 peptides to the surface of PEG-b-PCL polymersomes	*Benefits:* Specific binding to the outer hair cells within the cochlea. Limitation: Limited longitudinal follow up to monitor long term functional status of inner ear.
Zhang et al.[128]	In vivo (Rat)	Transtympanic injection and cochleostomy	PEG-*b*-PCL polymersomes modified with Tet1 peptide	*Benefits:* Provided target binding site to cochlear nerve. *Limitations:* ochleostomy approach to deliver nanoparticle is invasive and may worsen the existing pathology
Xu et al. [102]	In vivo (Mice)	Postauricular hypodermic injections	The gentamicin (GM) loaded into hollow mesoporous silica (HMS) coated with uniformed zeolitic imidazolate framework (ZIF) nanoparticles (GM/HMS@ZIF)	Benefit: 1) Good biocompatibility and cellular uptake. 2) A potent strategy for the controlled and sustained release of gentamicin for the treatment of MD. *Limitations:* Mouse model. Different inner ear structure and function to humans.
Xu et al.[129]	In vivo (Mice)	Intraperitoneal	ZIF-90 nanoparticles loaded with methylprednisolone (MP)	*Benefits:* 1) A safe vehicle for delivery of ototoxic drugs with appropriate protection against noise. 2) Negligible damage to the inner ear. 3) Low nephrotoxicity during treatment.
Schmidt et al.[130]	In vitro (Rat)	Install on implants	Brain-derived neurotrophic factor (BDNF) Nanoporous encapsulated in silica nanoparticles (NPSNPs), with a diameter < 100 nm	*Benefits:* Effectiveness of NPSNPs in transporting BDNF into the inner ear for protection of spiral ganglion neurons.
Ramaswamy et al.[91]	In vivo (Mice)	Intraperitoneally	Steroid-loaded SPIONs nanoparticles magnetically are delivered to the cochlea of the mice treated with cisplatin	*Benefits:* Protection of outer hair cells from cisplatin-induced ototoxicity. *Limitations:* safety has not been evaluated.
Leterme et al.[88]	In vivo (Wistar rats)	Magnetic field	Transversing the RW by an external magnetic field for administeration of SPIONs into the cochlea	*Benefits:* SPIONs can be administrated via RWM up toward the cochlear apex (low-frequency region), without hearing loss. *Limitations:* An immediate but transient hearing loss can be observed at high frequencies.

Table 2 (*continued*)

Reference	Study model	Administration method	Strategies	Benefits/shortcomings
Shimohi et al.[87]	In vivo (Rats)	Intratympanic	Intratympanic injection of steroid prednisolone loaded into SPIONs	*Benefits:* Limited adverse safety effects for treatment of cochlea through magnetic injection after comprehensive study on rodents. *Limitations:* Slight inflammation in cochlea.
Zou et al.[78]	In vivo (Rat)	Transtympanic injection	Employing different concentrations of silver NPs	*Benefits:* 1) Access to the inner ear for drug delivery. 2) Micro CT visualization of silver *Limitations:* Mouse model - different cochlear structure & tonotopic map to humans.
Kayyali et al.[63]	In vitro & vivo (Mice)	Intraperitoneal	Gold nanoparticles	*Benefits:* Enhancement of CT imaging of the inner ear. *Limitations:* absence of specific targeting to the outer hair cells.
Yang et al.[131]	In vitro (Mice)	Intratympanic injection	Dexamethasone loaded in phospholipid NPs	*Benefits:* 1) Enhanced recovery of hearing loss in comparison with Dex sodium phosphate (Dex-SP) solution. 2) Stronger anti-inflammatory effects than Dex-SP. *Limitations:* Mouse model limits direct translation (i.e. different size of cochlea and concentration gradient (pharmacokinetics)).
Cervantes et al. [105]	In Vitro	Intracochlear	Encapsulation of dexamethasone and hydrocortisone into Solid lipid nanoparticles (SLNPs)	*Benefits:* Increased survival of organ of Corti cells in an ototoxic experimental model after treatment with SLNPs encapsulated steroid drugs. *Limitations:* in vitro model results in poor translation to in vivo homeostatic environment and clinic.
Wang et al.[106]	In vivo (Guinea pigs)	Intravenous and intraperitoneal administration	Clozapine solution or sulphate SLNP was administered though intratympanic or intravenous injection	*Benefits:* Halts production of Reactive Oxygen Species (ROS) in the cochlea after noise. *Limitations:* Reduced hearing thresholds, measured via ABR.
Lajud et al.[93]	In vivo (Mice)	Intraperitoneal	Encapsulation of liposomal NPs into a CS-based hydrogel	*Benefits:* Controlled and sustained delivery of different drugs into the inner ear without damage

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Table 2 (*continued*)

Reference	Study model	Administration method	Strategies	Benefits/shortcomings
				to the sensory structures. *Limitations:* The concentration of intact liposomes within the organ of Corti has not been quantified.
Kechai et al.[132]	In vitro In vivo (Guinea pigs)	Transtympanic injection	Dexamethasone phosphate (DexP) loaded into a hyaluronic acid (HA) gel combined with PEGylatedliposomes	*Benefits:* Sustained delivery of corticoids to the inner ear by administrating HA liposomal gel to the middle ear. *Limitations:* 1) System safety has not evaluated. 2) In vitro work has limited translation.
Kim et al.[133]	In vitro In vivo (Mice)	Intratympanic administration	DEX-loaded PLGA NPs based on thermosensitive hydrogels	*Benefits:* 1) No observed cytotoxicity, even at a concentration of 4 mg/mL. 2) Sustained release of DEX-NP-gels in the middle ear after IT administration. *Limitations:* Mouse model and in vitro work limits clinical translation.
Kayyali et al.[134]	In vivo (Mice)	Intraperitoneal	c-Jun N-terminal kinase (JNK) inhibitor payload incorporated with CS glycerophosphate (CGP)-hydrogel as targeted and multifunctional NPs	*Benefits:* Protection of outer hair cells from noise induced hearing loss by delivery of NPs and their 'payload'. Limitation: Targeted liposomes are also localized in off-target tissues.
Lin et al.[135]	In vivo (Mice)	Ultrasound microbubbles	Delivery of CS-coated gold nanoparticles by means of ultrasound microbubbles	*Benefits:* Incorporation of USMBs with CS-AuNPs as efficient drug and gene delivery methos for inner ear therapy. *Limitations:* Mouse model.

inner ear between one and three hours.[30,33,145] Given the delicate and vulnerable structure of the inner ear, NPs must possess a high capacity for the loading of drugs and be non-toxic and biocompatible to avoid damage to the sensory structures. They also must have controllable drug release kinetics at their targeted location at an adjustable time interval. The routes that NPs can be administrated into the cochlea are classified into local administration through Intra-Tympanic (IT) injection, gel-foam application to the Round window membrane (RWM), magnetic transfer via iron oxide NPs added to the RWM, direct injection, or transtympanic injection onto the oval window.[35] Further to this, Ding et al.[32] demonstrated that the direct or transtympanic injection of nanocarriers into the oval window could greatly assist in the specific targeting of the vestibular system to treat balance dysfunction associated with MD. Various nano-based drug delivery systems that have been employed or hold the capacity to be used in treating MD can be categorised in the following sections below.

Nanogels

Nanogels are a group of NPs characterised by their high water content. They are considered a very adaptable drug delivery strategy, particularly for targeted and controlled delivery of therapeutic factors.[146] Hydrogels can be used for improved pharmacokinetics, [147] as it is demonstrated in [148], where the use of hydrogel led to a sustained release of gentamicin for MD treatment, and localized ototoxicity in the hair cells of the cochlear and vestibular systems. The combination of hydrogels with NPs provides a targeted and sustained delivery system, which has the potential to overcome the drug delivery problems associated with the inner ear, such as, the short half-life of drugs in the cochlea and their rapid elimination.[39,149] For instance, in

previous work conducted by Dai et al., a protein drug was delivered locally to the inner ear by loading PLGA NPs in a thermosensitive hydrogel.[92] It was demonstrated that the drug release rate was slowed by 1.5 to 3-fold in the nanogel drug delivery system.[92] This was in part due to a higher viscosity of the drug, NP and hydrogel interaction at body temperature.[150]

Similarly, Polyethylenimine (PEI)-based nanogel NPs can be situated on the RWM to achieve a slow and targeted drug delivery. A method of cross-linking via star-shaped PEGs can also boost the stability of the gel.[21] To enhance the delivery of drugs by nanogels, they can be made to degrade upon cytosolic entry, or they can transfer hydrophilic charges, including peptides and nucleic acids. They can also be used as tracers both in vivo and in vitro (fluorescent dyes) by attaching them to trace factors.

Polymeric Nanoparticles

Polymeric NPs are systems comprised of synthetic and natural polymers. They provide substantial benefits compared to other nanocarriers, like micelles, liposomes, and inorganic nanomaterials, and include scale-up feasibility and various synthesis methods.[151] Other unique properties of polymeric NPs include their good stability in fluids, along with the functionalisation of their surfaces, extensive accessibility of various polymers, and the ability to modulate their degradation as a function of detailed stimuli.[152] Most of the polymers with FDA approval are multi-unit polymers, and PEGylated drugs.

Polymeric NPs have helped produce a wide array of drugs, including hydrophobic and hydrophilic substances and biomolecules, like peptides and proteins, at a decent loading capacity. Furthermore, they can carry visualization agents like SPIONs and fluorescent quantum dots.[21]

PLGA and PEG are among the most efficient drug delivery systems that can improve the half-life of molecules in circulation, as well as their biocompatibility.[151] PLGA nanoparticle is an FDA-approved biocompatible and biodegradable polymeric NP, which has been extensively investigated for drug delivery.[107] The surface of these NPs can be modified by various targeting moieties, providing them with targeting capability.[153] PEG is a synthetic polymer that can be directly conjugated with drugs or can be used on the surface of nanomaterials as a stealth coating to evade the immune system and enhance half-life circulation.[154] For targeted drug delivery purposes, PEG can be utilized to conjugate targeted ligands or peptides onto NPs and bind the corresponding receptors on the surface of cells.[155] PEI is a cationic polymer utilized as a co-delivery system for various types of combinations and can enhance the internalization of drugs.[156] It is also demonstrated that PEI can enhance functionality and gradual release of some drugs.[157] However, the cytotoxicity of PEI is a point of concern.[156]

It has also been shown that PLGA NPs have more uniform distributions throughout the inner ear as compared with other delivery strategies. The proper distribution of PLGA-encapsulated iron oxide within the inner ear, including various membranes, hair cells and supporting structures, was demonstrated by Ge et al.[158] In most studies, it is asserted that PLGA nanocarriers can keep their integrity in the perilymph[34,145], and their maximum concentration in the RWM was witnessed 30 minutes after transtympanic injection.[2] In a study the ability of PLGA NPs in simultaneous encapsulation of multiple agents is investigated. A combination of three kinds of drugs, including notoginsenoside, ginsenoside Rg1, and ginsenoside Rb1, demonstrating potential in protecting the spiral ganglion cells from cochlear ischemia, were loaded into the PLGA NPs. The results revealed that a higher dosage of drugs were found in the perilymph of guinea pigs than delivery in a free solution which supports PLGA NPs can be considered as a powerful drug delivery method across the RWM.[124]

Dexamethasone is commonly used as a medicine for MD treatment[5] and can be delivered into the inner ear by PLGA.[94] It has been demonstrated that dexamethasone-loaded PEG-coated PLGA particles can improve cisplatin-induced ototoxicity, following local administration of the drug into the cochlea in guinea pigs.[94,125] Additionally, Sun et al.[125] demonstrated that the administration of dexamethasone by PLGA NPs led to a more sustained release, associated with improved outcomes over time. Furthermore, the prodrug form of the compound needs to be converted into its active form for effective drug delivery. While the prodrug dexamethasone phosphate is received by the control group, its active form is encapsulated by NPs and nanoemulsions.[125,131] However, by measuring dexamethasone in its active form within tissues, Yang et al. revealed that the concentration of dexamethasone in either dexamethasone-loaded nanoemulsions or dexamethasone phosphate was similar, revealing significant conversion from prodrug to active form using this method.[131] Kuang et al., via a zebrafish model, demonstrated that SS-31 peptide-conjugated geranylgeranylacetone-loaded PLGA NPs can efficiently protect hair cells against gentamicin-induced damage.[126]

A study conducted by Ding et al.[32], employing near-infrared fluorescence imaging revealed that chitosan nanoparticles (CS NPs) encased in the poloxamer 407 thermosensitive gel can enter the vestibule of the inner ear through the oval window after intratympanic injection in a guinea pig experimental model. The results of the study demonstrated that the oval window can be considered a more impressive gateway than RWM for the delivery of CS NPs into the vestibule.[32]

Polymersomes, called multifunctional NPs, are another group of polymers whose outer shells are assembled by amphiphilic block copolymers. They have the capability to carry hydrophilic and hydrophobic drugs (e.g., dexamethasone) by encapsulating them in their core and membrane, respectively.[2] It is also possible to raise the cellular uptake of hydrophobic and hydrophilic molecules by functionalising them with a variety of molecules. Poly (ε-caprolactone) -bpoly (ethylene glycol) PEG-b-PCL can be used to form polymersomes, which is an FDA-approved polymer, forming a hydrophobic core. PEG, which contains a polymeric hydrophilic crown, due to its compatibility and resistance to protein uptake and cell adhesion, leads to a longer circulation time of the polymer in the body.[159]

PEG-b-PCL polymersomes modified with various peptides, specifically, A665 and A666, have shown good outcomes in targeting outer hair cells within the inner ear.[127] PEG-b-PCL polymersomes with Ten-eleven translocation methylcytosine dioxygenase 1 (Tet1) peptide provided a target binding site to specific cells inside the inner ear. In fact, the Tet1 peptide, found on neurons, is anticipated to target the polymersomes on the

cochlear nerve. The Tet1 modified PEG-b-PCL polymersomes were administered by cochleostomy, resulting in effective cochlear nerve targeting. This has promising use for drug delivery for the treatment of neural dysfunction associated with inner ear disease, like MD.[128]

Asymmetrical polymersomes with properties such as type of membranes and great watery cores can also encapsulate different hydrophilic and hydrophobic drugs. Their stability and tunable membrane formulations make them appropriate for effective drug delivery. However, more studies are needed to improve the loading capacity of drugs, increase the efficiency of controlled release, and enhance the in vivo circulation half-life to target drug delivery to the inner ear.

Inorganic Nanoparticles

Another drug delivery system is based on inorganic nanoparticles that are very popular for the delivery of drugs to the inner ear because of their availability, biodegradability under physiological conditions, small size along with antimicrobial and magnetic properties.[160] Mesoporous silica nanoparticles (MSNs) are a kind of inorganic NPs that can encapsulate drugs within their pores, leading to the controlled release of antimicrobial platforms.[161,162] In a recent study, it was shown that loading gentamicin into hollow mesoporous silica and coating the surface with zeolitic imidazolate framework (ZIF) NPs can act as a good delivery system. This approach may provide the opportunity for constructing a vehicle for delivery of ototoxic drugs to control vertigo attacks in MD and improve gentamicin treatment, via the controlled and sustained release of low-dose drugs.[102] The capability of MSNs in controlling the encapsulation and release of antibiotics revealed that these NPs have great potential in the treatment of infectious disease within the inner ear.[163]

Metal-organic frameworks (MOFs) are another porous nanoparticle demonstrating a great capability to carry drugs into the inner ear. Encapsulating Methylprednisolone (MP) into the ZIF-90 NPs was employed successfully to treat Noise-induced hearing loss (NIHL) as an inner ear disorder.[129] These NPs exhibit better protection against noise in comparison to free MP and MP@ZIF-8, as well as low damage to the structure of the inner ear, and low nephrotoxicity.

Other nanomaterials, such as porous silicon, can also be used as a nanocarrier for drug delivery. They have various advantages, including ease of synthesis, biocompatibility, low toxicity and degradability, which is appropriate for their use in targeted drug administrations for inner ear disorders, like Meniere's disease.[164] Porous silicon particles act as dispersed transport systems in the intravenous administration of drugs. This was evaluated through the amount of the autotropic effect following gentamicin sulphate administration. The autotropic effect of the pharmaceutical marker of cochlea accessibility was demonstrated when applying porous silicon particles with a size of about 600 nm. However, 600 nm is generally too large to be classified as a nanoparticle and this diameter is associated with decreased passage into the round window and inner ear tissues. The detected autorepression in the simulation of ototoxicity shows improved permeability in the inner ear.[164] Nanoporous silica NPs can also be applied in the treatment of inner ear disorders. Spiral ganglion neurons can be targeted, and loaded with a brain-derived neurotrophic factor (BDNF) that is released in the longterm.[130] Additionally, BDNF has also demonstrated effectiveness in otological outcomes related to cochlear implantation, which may be used in MD patients who are candidates for this treatment modality.[165]

The active surface of silica NPs is capable of functionalisation to altered surface properties and loaded therapeutic factors. Other characteristics of silica NPs include loading capacity, pore size, and porosity, which can be changed according to the materials applied to their synthesis. Silica NPs with a size of about 20 nm are safe to apply to the inner ear. Moreover, NPs with a size of <100 nm are agreeable to the integration into various materials, such as polymers or hydrogels, and are beneficial for the production of cochlear implant-related delivery systems, such as electrode coatings. In spite of various studies using silica NPs to control drug delivery, their long-term toxicity profile and accumulation in tissues remain a relatively uncharacterized and a challenge. It is important to pay attention to immune responses after the administration of silica NPs. Furthermore, the size of NPs, synthesis method, functionalisation with biocompatible molecules, surface charge (less negative charges look to suppress the immune response) and porosity (with increasing porosity of NPs, hemolysis of red blood cells occurs less), are important parameters to consider when designing silica NPs with low toxicity and reduced inflammatory reactions.

SPIONs are another main category of inorganic nanoparticles with a small size of 5–15 nm. These NPs can permeate the RWM and reach the cochlear tissues driven by magnetic fields.[89,140,141] Although, SPIONs are not capable of encapsulating any drugs, they can be coated with drugs or loaded into polymeric NPs, such as chitosan nanocarriers and PLGA NPs.[2,91,140,141] For instance, dexamethasone is used to treat inflammatory inner ear disorders, such as MD, and has further potential to improve as a therapeutic candidate.[166] Notably, administrating the combination of dexamethasone and SPIONs into the perilymph via the RWM demonstrates no toxicity in animal models such as rats and guinea pigs.[86,87] In this combination, dexamethasone was coupled with 500 nm PLGA NPs containing SPIONs. By this method, dexamethasone was shown to permeate the RWM and disperse into the inner ear; its distribution was facilitated by a contralateral magnetic field of 0.26 mT.[86] However, since the distance between the magnetic field and the NPs must be <2 cm in humans, this method needs improvement.[86] Sarwar et al.[167] proposed a system of four magnets that allowed larger distances of between three to five cm by creating more control of NPs in the inner ear.

The safety of employing a magnetic field as a drug delivery tool has been examined widely. Although in most cases there were no serious adverse effects,[87,168] mild inflammation was reported in some studies, which needs further clarification.[87,91,169] Shimoij et al.[87] extensively evaluated the safety of magnetic delivery within three months after the injection of the initial dose (Fig. 2). A major limitation of this technique is the potential for iron oxide to accumulate within the inner ear, which may be associated with inner ear dysfunction and hearing loss.[169]

The size of iron oxide NPs including cores, coatings, type of core, concentration, and type of synthesis method have a certain impact on their biocompatibility, affecting cell and tissue behaviors. Green synthesis demonstrates low toxicity and is safer than other methods, meaning it is an acceptable method to produce metal and

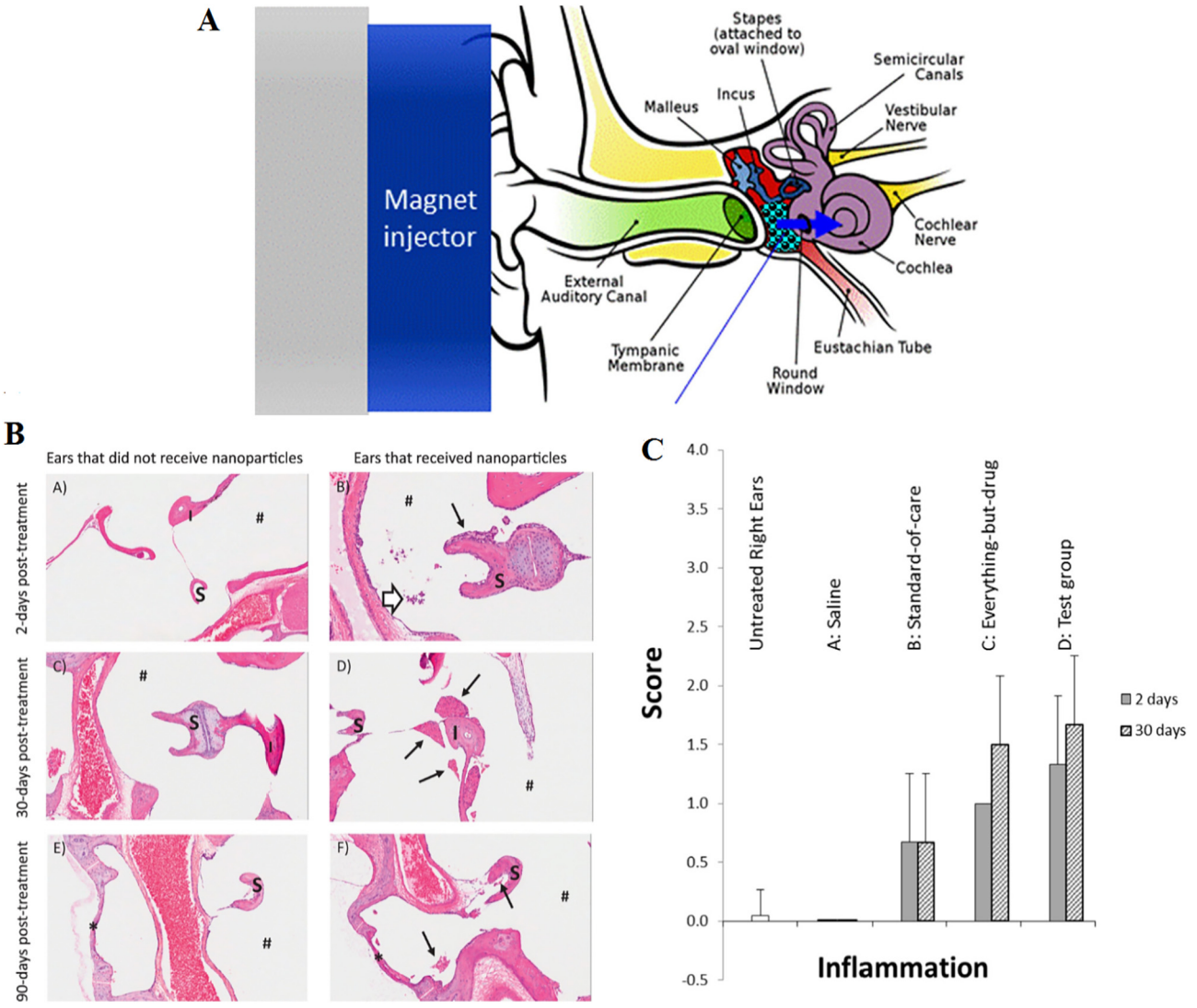

Fig. 2. Schematic illustration of the injection of magnetic NPs to the cochlea (A); Histological examination of middle ear structures in the control situation (no nanoparticles) (left column) or following nanoparticle administration (right column). Importantly, there was low neutrophil count and macrophages observed 48 h after NP treatment (B); Mean inflammation histopathology scores for treatment vs controls (C).[87]

metal oxide NPs, such as SPIONs. On the other hand, polymers have better biocompatibility for the coating of iron oxide NPs. The application of polysaccharides, PEG, PLGA and their copolymers as a coating can increase their stability and safety. The NP–protein corona complex could improve safety, distribution, and blood circulation of SPIONs. These particles comprise of various protein layers and are able to alter the properties of NPs.[170] They could have various physico-chemical properties depending on the size and surface of SPIONs, which makes them practical for permeability across barriers, such as RWM or Blood-brain-barrier (BBB). During the time that protein uptake occurs, proteins undergo structural rearrangements that lead to changes in NPs levels, providing promise in the treatment of inner ear diseases to reduce the toxicity of iron oxide NPs.

Lipid Nanoparticles

Lipid NPs are another delivery system that has been investigated for use in the inner ear.[105] These particles can be categorised as solid lipid NPs (solid core), liposomes, lipid nanocapsules (liquid core), and nanoemulsions (oil nanodroplets within an aqueous phase). They are considered an attractive drug delivery tool because of their biodegradability and capacity to deliver hydrophilic and/or lipophilic drugs.[105,106,131,171,172] Due to the fact that solid lipid NPs (SLNPs) are solid at body temperature, there will be better control on drug delivery in comparison to liquid systems.[105] Indeed, the mobility of drugs in a liquid form is much more than in the solid state, which causes a rapid release of drugs. This feature leads to using higher doses of drugs that increase the risk of toxic side effects.[173] Amphiphilic liposomes can convey their cargo through the RWM and transport it to targeted cells within the cochlea.[141] Phospholipid-based NPs can encapsulate both hydrophobic and hydrophilic molecules in the phospholipid bilayer and aqueous core, respectively. Dexamethasone, which is a lipophilic drug, can be encapsulated in lipid-based nanocarriers.[103] The uptake and targeting of drugs can be modified in lipid-based NPs through changes in the surface charge and hydrophilicity.[107] Encapsulating

dexamethasone in phospholipid NPs, regardless of their type (neutral, anionic, cationic, and cationic-PEG), has great capacity for hearing recovery, due to the effective penetration and distribution of the drug into the inner ear; however, cationic-PEG NPs (Cat-PEG NPs) had the most desirable result and were the only type that demonstrated remarkable cellular uptake into cochlear structures, such as the organ of Corti.[131] A protective anti-inflammatory effect was achieved by delivering dexamethasone to the mouse RWM via Cat-PEG NPs.[131] Notably, dexamethasone was effectively delivered to cochlear hair cells by the Cat-PEG NPs, gaining good therapeutic results (Fig. 3).

Cubosomes are another group of highly stable, self-assembled NPs that arise from a lipid cubic phase, demonstrating the capacity for improved drug delivery.[174] The more effective and larger membrane surface area of the cubosomes in comparison to liposomes allow enhanced hydrophilic and lipophilic drug loading.[2,175] The composite of cubosomes can be adjusted to modulate pore sizes, for greater flexibility and stability of use under physiological conditions.

Lipid NPs are effective drug delivery systems which have various benefits compared with metal-based and polymeric nanocarriers. The most significant benefits of them are their degradability, biocompatibility, less immunogenicity, controlled release and ease of use. They can be administered by various assays include oral, topical, ocular, and inner-ear administration. With relevance to the current coronavirus disease of 2019 (COVID-19) and established mRNA vaccines, lipid NPs have been a key element in efficiently protecting and transporting mRNA to desired cells. With their success, lipid NPs will likely assist with the advances of novel gene delivery systems and gene editing-based treatment of various diseases, including debilitating inner ear conditions.

Combination of Nanoparticles With Other Drug Delivery Techniques in MD

The combination of NPs and hydrogels provides an adaptable hybrid system with appropriate properties for delivering a vast number of drugs into the inner ear to treat disorders such as MD. Pharmaceuticals involving hydrogels have gained significant attention recently for their drug delivery advantages related to the inner ear. For example, they can hold a great deal of water, and offer stable state drug release over days to months. Furthermore, they can deliver proteins and small molecules, and are completely absorbed in delivery. Lajud et al.[93] demonstrated that nanohydrogels are successful as a controlled and sustained delivery systems for transferring therapeutic compounds in stable NPs to cellular structures within the labyrinth. According to this study, liposomal NPs demonstrate ongoing stability for more than two

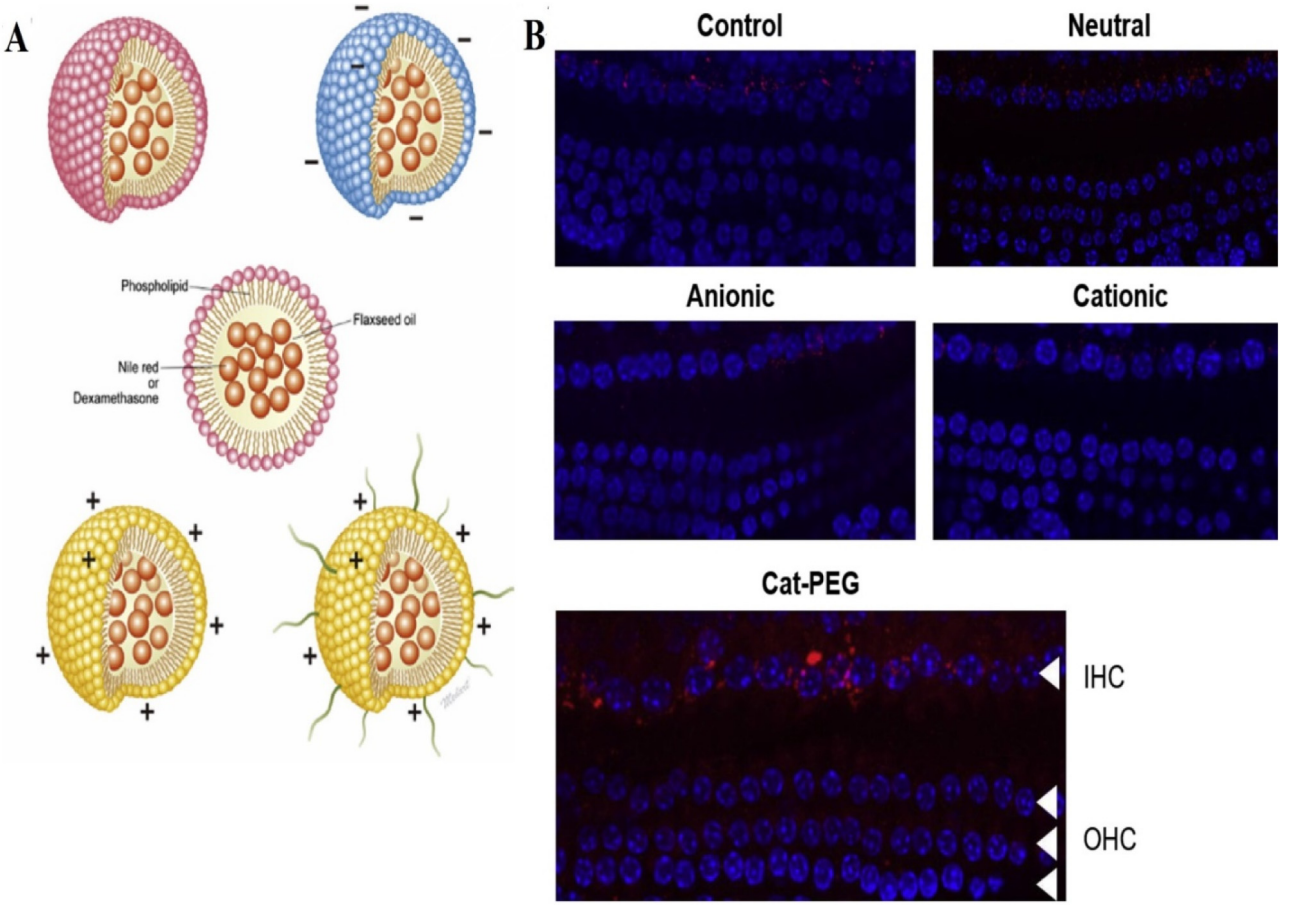

Fig. 3. Schematic illustration of the different NPs synthesised from phospholipid nanoemulsions (A); *Differential effects of PEG administration on cochlear hair cell function within the organ of Corti (B).*[131]

weeks under physiologic conditions in vitro, and the chitosan-glycerophosphate-hydrogel-nanoparticle system (nanohydrogel) delivers these nanoparticles in stable form via the RWM into the perilymphatic spaces of the labyrinth. Consequently, their 'payload' such as dexamethasone or gentamicin can be delivered to inner ear structures, making them a suitable treatment candidate for MD.

Since liposome NPs in the hyaluronic acid gel can remain in the inner ear for a long time, loading prodrug dexamethasone phosphate onto the liposomes and depositing it into hyaluronic acid gel creates a local source of drug adjacent to RWM. It has been shown that over 30 days, this leads to an improved and sustained release of dexamethasone into the perilymph, with a higher conversion rate of dexamethasone phosphate (prodrug) to dexamethasone (active drug).[93,132]

Moreover, it has been demonstrated that the retention time of drugs within the cochlea can be enhanced by loading PLGA NPs into a chitosan/glycerophosphate-based thermosensitive hydrogel, compared with the administration of PLGA NPs alone.[92] Chitosan is a non-toxic and biodegradable polymer, which has promising utility to treat inner ear disorders. This polymer can encapsulate hydrophilic drugs, such as dexamethasone, and deliver these drugs in a controlled and sustained manner from the middle ear to the inner ear.[93] The combination of chitosan and PLGA can increase the dosage of drugs delivered to the perilymph for local administration.[92] Hydrophilic molecules such as poloxamer are other options that boost the efficiency of PLGA NPs with regard to cellular uptake through surface modification.[176] In a recent study conducted by Dong-Hyun et al.[133] DEX-loaded PLGA NPs based on poloxamer hydrogels were developed in order to present a sustained drug release, and it was shown that more than half of the initial dose of dexamethasone remained in the middle ear for up to two days. Moreover, no cytotoxicity of DEX-NP-gels was observed illustrating the potential use of them as new drug delivery methodologies.[133]

NPs loaded in hydrogels, such as chitosan–glycerophosphate, can be used in delivering corticosteroids and aminoglycoside antibiotics (such as gentamicin) into the inner ear perilymph.[134,177] Importantly, a reduced risk of auditory dysfunction and hearing loss has been associated with this method, compared to drugs delivered via IT injection.[178] Moreover, nanohydrogel-treated ears revealed improved colocalisation when compared to NPs by themselves, hence demonstrated better delivery of structurally intact NPs and their 'payload' to cellular structures of the inner ear (such as hair cells and supporting cells). Overall, these findings demonstrate nanohydrogels are an effective and controlled method to deliver NPs to the inner ear for treatment.

The combination of hydrogel with NPs could overcome the toxicity of NPs and decrease other associated risks. Indeed, in spite of the great developments in NPs technology, some limitations still remain. For example, in systematic delivery of NPs, unwanted exposure of some payloads can cause toxicity in some organs.[179] Moreover, in local delivery, some NPs may drain away from the targeted injection site, or some NPs' payloads can release prematurely and affect the untargeted sites or the surrounding environment[180,181]. Albeit, the NPs toxicity is highly dependent on the physical and chemical properties of them, and the mentioned issues can compromise the safety of employing NPs.[182] Combining NPs and hydrogels in a single platform can alter the limited features of each component, enhance the stability of drug payloads and avoid their premature release. The NPs-hydrogel platforms can be created in a wide range of structures because of the vast availability of nanomaterials and gel matrices[183] and the physical or chemical interaction of NPs with polymer chains and other NPs resulting in creation of crosslinks and gel-like structures, respectively. Based on the research conducted in,[184] the conjugation of porous silicon NPs with gold nanorods and encasing them in hydrogels can alleviate the drug leakage and provide a system with more controllable releasing capacity.[184] Moreover, Chen et al. in a comparative research revealed that incorporation of metal-organic framework (MOF) NPs with some kind of hydrogels can decrease the leakage of the loaded drug and increase loading efficiency.[180]

Furthermore, various combinations of these two types of materials demonstrated enhanced mechanical strength, electrical and thermal properties, as well as stimuli response. Various physical properties of the nanocomposite hydrogels can be adjusted by manipulating the chemical structure of the hydrogel, such as, the diffusion coefficient or swelling ratio, which may affect the release behavior of drugs/genes from the hydrogel. With regards to the local delivery of pharmacological agents, nanocomposite hydrogels can act as a pool of drug molecules based on the reaction among drug, the molecule and the hydrogel matrix field. Nanocomposite hydrogels are also good candidates with regards to the development of wearable devices as well as implantable drug delivery microsystems, as they are flexible and safe. This promises novel creation of devices, with future applications in preclinical and clinical tools.

Nanoparticles and Ultrasound techniques have great potential and future use in the treatment of MD. Ultrasound is a relatively new and effective strategy for drug delivery to the inner ear.[185,186] In a recent study, the combined use of ultrasound and nanocarriers provided sustained release of drugs and enhanced the concentration of dexamethasone phosphate within the inner ear. In that study, Shih et al. demonstrated that surgery is needed for ultrasound drug delivery, as opposed to magnetic delivery which can be a drawback of this method.[186] Nonetheless, Liao et al.[185] developed a new approach for delivering drugs by the use of ultrasound-induced microbubble (USMB) to avoid the need for surgery. Here, it was shown that this method enhanced gentamicin uptake and gentamicin-induced hair cell loss, which can be used for nanocarriers administration. To this end, the ultrasound-induced techniques lead to both formation of monodispersed NPs-based carriers and also inducing the specific cells inside the inner ear. Recently, Lin et al. studied the impact of using USMB on enhancement of RMW permeability delivery of chitosan-coated gold nanoparticles (CSAuNPs) into the inner ear.[135] It demonstrated that transient disruption of the outer epithelium barrier results in efficient delivery.

Novel methodologies based on the convergence points between the chemistry, physics and biology can change difficult problems to practical opportunities. The sound-based techniques are considered as one of the wave-based methods for both of the preparations/synthesis and applications approaches. These types of methods can lead to lowering the size distribution of any types of particles, and also increasing their surface active sites. Therefore,

by increasing the surface active sites, the possible targeted accumulations on the surface enhanced, and also improving the efficiency of different types of the surface-based methods even for the passive/active targeting.

Another system which has recently developed to overcome the biological barriers of the ear is known as Microshotgun (MS).[187] It can be deemed as a biocompatible transmembrane delivery system which is able to cross the tympanic membrane (TM) and RWM and propel NPs through these membranes. NPs can be loaded into the MS and empowered in two stages. In the first step, NPs can penetrate the epithelial layer of TM. Then, using an external magnetic field can lead them to penetrate the TM endothelial layer. The constructed tool has illustrated great improvement in delivery of NPs. It is demonstrated the micro-penetration caused by MS was healed completely within 24 h which is extremely low in comparison of other treatment techniques. As well as this, no toxicity was witnessed for this device during the examined period.[188]

It can be seen the combination of nanoparticles and other novel techniques such as ultrasound and MS can meet the challenges facing inner ear disorders like MD to some extent.

Clinical Studies

Sensorineural hearing loss, as one of the most prevalent sensory deficits effecting humans, has an unknown etiology, especially in the context of complex inner ear disorders such as Meniere's disease. Moreover, the corresponding therapeutic alternatives are restricted due to a lack of both understanding regarding inner ear pathophysiology and effective medications and non-invasive targeted delivery approaches to the inner ear.[39] Regarding drug delivery systems, systematic and local administration routes are employed to mitigate the related symptoms of inner ear disease. On the one hand, while ease of administration is a desired characteristic of systematic delivery, it shows undesirable adverse effects such as higher systematic dose to reach the therapeutic level (due to limitations caused by the BLB or limited blood supply).[39,143] On the other hand, IT administrations can bypass the BLB and labyrinthine arterial supply, but drug clearance through the eustachian tube is a shortcoming of this delivery system, leading to probable sub-therapeutic dose levels.[39,189] Further, the intracochlear (IC) administration, as another local delivery route, provides higher drug bioavailability, while raising the risk for permanent hearing loss and vestibular damage, with additional challenges such as how to effectivel get the drug into the sensory cells.[190,191]

NP-based delivery systems have potential to tackle these challenges. Nowadays, it has been demonstrated that several types of NPs might be appropriate for drug delivery to the inner ear for diagnsotic (e.g., imaging) and treamtner purposes. Although nanoparticle-assisted delivery systems have come along way and established a new pathway to treat inner ear disease, much more progress is needed before these candidates can be used in a clinical setting.[192] Most of the existing works on employing NPs for inner ear drug delivery have focused on animal models. However, there exist few clinical trials investigating NP-based delivery systems for diagnosis or therapy. One of the barriers to corresponding clinical investigations is the-possible ototoxicity and damage to the sensory end organs within the inner ear, which should be taken into consideration prior to use in human studies.[193,194]

Nano-based technologies, like nanoparticles, dendrimers, polymersomes, liposomes, and SPION nanoparticles, hold enormous promise for safe and targeted delivery to the inner ear. Drugs are conjugated to NPs, producing nanoformulations. Nonetheless, the liquid nature of the structures causes low bio-retention. Hydrogel systems, among various approaches, have the ability to achieve a high bio-retention of nanoformulations and prevent their clearance through the eustachian tube.[190,195] Different clinical and preclinical studies are being done on a range of hydrogel-based formulations (Poloxamer 407, chitosan, hyaluronic acid, and biodegradable block copolymers).[107,189] Poloxamer 407 conjugated with dexamethasone was studied in phases II (NCT02997189) and III (NCT02612337) for cisplatin-induced hearing loss and Meniere's disease, respectively; however, they have recently been terminated due to negative efficacy outcomes from the Phase III study 104–201,506. In addition, formulation with gacyclidine is in a phase II clinical trial (recruiting; NCT04829214) for tinnitus and with ciprofloxacin completed phase III (NCT02600559) for otitis media. In addition, other nanoparticles and nanoformulations have been considered less for human studies.

Overall, this field of research is of great interest in developing novel delivery systems which are minimally invasive, highly targeted, and controllable. Additionally, in the context of diagnosis, NPs, particularly gold nanoparticles and SPIONs, have illustrated potentials in inner ear imaging, and more focused studies are required to perform so as to decipher their hidden capabilities. It is predicted that there will be more clinical trials using less-toxic nanocarriers for imaging and therapeutic purposes, particularly for MD treatment.

Future Perspective and Challenges

The functionality of nanocarriers in the treatment of inner ear disorders like MD has undergone rapid progress within the last decade, particularly in synergy with other strategies. Some kinds of NPs enhanced the accuracy of MRI and CT tools in diagnosing MD, which can improve patient outcomes throughout disease progression. Moreover, novel NPs have great impact on the delivery of drugs in more sustained ways and compensate for some drug properties, including poor solubility, degradation, low half-life as well as restricted passage across physiological barriers. However, there are some concerns regarding the employment of NPs which should be addressed in the future. Zhang et al.[196] demonstrated that some dosage-dependent toxicity was observed in the targeted cells treated with lipid core nanocapsule (LCN). The surviving rates of treated cells were: 37.94, 86.41 and 80.06 % for 1.5, 0.15 and 0.015 mg/ml LCN concentrations, respectively. Concentration-dependent nanotoxicity was also observed in [197]. The auditory brainstem response revealed that AgNPs-induced hearing loss can be partially recovered or be reversible based on the NPs dosage. Nano-toxicity to the cochlear structure and damage to the cultured cochlear epithelium of neonatal mice was observed by using excessive weight ratio of linear polyethylenimine (L-PEI) and plasmid DNA for gene

therapy.[198] In an in vitro study, a size-dependent nanotoxicity was witnessed for SPIONs, where the number of survival cells was lower in using NP-100 nm in comparison to 500 nm.[168]

It can be seen that some physiochemical features of the nanoparticles like particle size, shape and surface-features can play a role in their ototoxicity. Therefore, further research should be done to define the role of these parameters on ear toxicity.

Furthermore, most of the provided information about the ear toxicity of NPs is acquired based on the experimental models and information about the side effects of nanomaterials on humans is lacking. Nanomaterials can have hazardous effects on patients, and those who work with nanomaterials for synthesis, production, environment, and so forth.[199] Therefore, future studies are required to focus on these aspects of nano-based drug delivery and treatment methods.

Besides, regarding MD treatment through the use of nanomaterials, it is seen that although several pieces of research have focused on the treatment of inner ear disease by nano-based materials, limited numbers of research projects have particularly addressed MD. There is a crucial need in this field, and researchers should perform greeather research on MD and the use of nanotechnology for diagnostic and therapeutic aims.

As clearly seen, SPIONs and PLGA, among others, have been used in the research related to MD. Moreover, two types of medicine including dexamethasone and gentamicin have been selected in the existing research. Therefore, other groups of nanomaterials, such PLGA NPs, magnetic NPs, lipid-based NPs, liposomes, polymersomes, and silica-based NPs, and other groups of drugs, which have shown great potentials for diagnosing or curing MD, are projected to be employed in the foreseeable future.

Finally, different properties of nanomaterials, including their efficiency, stability, cost-effectiveness, biocompatibility, and possible side effects, are to be analyzed for MD-based research, as done for other inner ear disorders, in vitro, in vivo, and also in clinical trials.

In this review, we have considered the current limitations in inner ear drug delivery, and the challenges of conventional drug delivery systems. We have investigated the most recent nano-based diagnosis methods and drug delivery systems, which provide promise for future use in inner ear disorders, such as MD. Notably, many novel nanodrug-delivery systems have been employed for several inner ear disorders, but have not yet been applied specifically to MD. Furthermore, many strategies have not obtained clinical use and are in early experimental stages. Although there is much progress to be made for NPs to be widely used in MD treatment, we are of the opinion that nanotechnology will ultimately present efficient solutions and facilitate clinical practice in the future.

CRediT authorship contribution statement

Afsaneh Kashizadeh: Data curation, Original draft preparation, Writing- Reviewing and Editing, Formal analysis.

Christopher Pastras: Writing- Reviewing and Editing, Validation, Investigation.

Navid Rabiee: Writing- Reviewing and Editing, Original draft preparation.

Masoud Mohseni-Dargah: Writing- Reviewing and Editing, Original draft preparation.

Payal Mukherjee: Reviewing and Editing, Intellectual discussion, Validation.

Mohsen Asadnia: Conceptualization, Writing- Reviewing and Editing, Resources, Supervision, Project administration.

Acknowledgements

Christopher Pastras was supported by a Macquarie University Research Fellowship (MQRF).

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