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## REVIEW

# Current strategies for drug delivery to the inner ear

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### KEY WORDS

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**Abstract** For many years, drug delivery to the inner ear has been a challenge to physicians in the treatment of inner ear disorders. In the past decade, the field of inner ear drug delivery has emerged with the development of new biomaterials and drug delivery technologies to improve the effectiveness of inner ear drug therapy. This paper reviews a number of inner ear drug delivery strategies including systemic, intratympanic, and intracochlear delivery. A focus of this review is the recent advances in intratympanic delivery of medications; approaches utilizing novel biomaterials as well as other recent developments are also discussed. Biotechnology-based approaches, such as gene and stem cell therapy methods are also reviewed. Among the various strategies, local drug delivery approaches including intratympanic and intracochlear drug delivery methods that limit systemic exposure are particularly promising. These inner ear drug delivery systems provide a new opportunity to improve the treatment of inner ear disorders.

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*Abbreviations:* BLB, blood labyrinth barrier; AAV, adeno-associated virus; ABR, auditory brainstem response; AIED, autoimmune inner ear disease; BDNF, brain-derived neurotrophic factor; BP, betamethasone phosphate; BBB, blood brain barrier; GDNF, glial-derived neurotrophic factor; GFP, green fluorescent protein; HBPL, hyperbranched poly L-lysine; LNCs, lipid core nanocapsules; MRP-1, multidrug resistance-related protein-1; PEG, poly (ethylene glycol); P-gp, P-glycoprotein; PEG-b-PCL, poly (ethylene glycol)-b-poly ( $\epsilon$ -caprolactone); PLGA, poly lactic/glycolic acid; RMW, round window membrane; ST, scala tympani; SGNs, spiral ganglion neurons; SSNHL, sudden sensorineural hearing loss; SPIONs, superparamagnetic iron oxide nanoparticles

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## 1. Introduction

Inner ear drug delivery has been a challenge to physicians in the treatment of inner ear disorders. In the past decade, new biomaterials and drug delivery technologies have been developed for inner ear delivery. Swan et al.<sup>1</sup> published a comprehensive review on inner ear drug delivery in 2008. In the same year, Borkholder<sup>2</sup> reviewed the status of inner ear delivery *via* the intratympanic and intracochlear routes. Since then, the field of inner ear drug delivery has gained a lot of interest and advanced rapidly. Several other review articles on inner ear drug delivery have been published in the past five years. Table 1 provides a list of these papers. These reviews focus either on a particular drug delivery strategy, a specific type of material or disease, or a combination of these topics in the field. The goal of the present review is to provide an updated general overview of inner ear drug delivery. We first review the administrative routes for inner ear drug delivery and then compare their advantages and disadvantages, discussing their potential as a result of recent advances in biomaterials, delivery technologies, and biotechnology methods. Recent developments in inner drug delivery strategies such as intratympanic and biotechnology-based approaches are highlighted. The strategies of inner ear drug delivery reviewed in this paper are listed in Table 2.

## 2. Anatomy relevant to inner ear drug delivery

To understand the challenges of drug delivery to the inner ear, the anatomy relevant to inner ear drug delivery is first discussed. The inner ear in humans consists of the bony labyrinth, a system of passages comprising two main parts: (a) the cochlea dedicated to hearing and (b) vestibular system

dedicated to balance. Blood labyrinth barrier (BLB) and round window membrane (RWM) are described below because these parts constitute the barriers of drug delivery to the inner ear.

### 2.1. Blood labyrinth barrier

BLB is a major barrier separating the inner ear from systemic circulation with tight junctions, made up of capillary endothelial cells that line blood vessels located in the stria vascularis<sup>13–15</sup>. It plays an important role in maintaining the microhomeostasis of the inner ear fluid and protecting the inner ear integrity similar to the function of the blood brain barrier (BBB) to the brain<sup>16</sup>. BLB functions not only as a physical barrier but also as a biochemical barrier with efflux pump systems, including P-gp (P-glycoprotein) and MRP-1 (multidrug resistance-related protein-1). These efflux systems further protect the inner ear<sup>17</sup>. Thus, the BLB is often considered as the rate-limiting barrier in the permeation of therapeutic agents from systemic circulation to the inner ear.

The current knowledge on the processes of drug transport through BLB in the literature is limited. The BLB consisting of tight junctions generally permits only the permeation of small lipid-soluble molecules<sup>1</sup>. Although the actual mechanism of tight junction opening of BLB is not clear, a number of factors were found to lead to tight junction opening of the BLB, including the presence of ototoxic drugs<sup>18</sup>, noises<sup>19</sup> and inflammation<sup>20</sup>. It has been reported that aminoglycosides given systemically in combination with diuretics such as furosemide or ethacrynic acid result in hearing loss and inner ear damage faster than that of aminoglycosides alone<sup>21,22</sup>. This suggests that the diuretics can enhance drug penetration across the BLB. Particularly, osmotic agents were suggested to

**Table 1** Review articles related to inner ear drug delivery.

Review subject of focus	Year	Author
Inner ear drug delivery applications and methods	2008	Swan et al. <sup>1</sup>
Transtympanic and intracochlear drug delivery	2008	Borkholder <sup>2</sup>
Pharmacokinetics of inner ear	2009	Salt and Plontke <sup>3</sup>
Clinical opinion on inner ear drug delivery and current status of inner ear disease treatment	2010	McCall et al. <sup>4</sup>
Cochlear implant	2010	Staecker et al. <sup>5</sup>
Nanoparticles	2010	Chen et al. <sup>6</sup>
Intracochlear drug delivery	2011	Borenstein <sup>7</sup>
Biodegradable materials	2011	Nakagawa and Ito <sup>8</sup>
Protective agents against sensorineural hearing loss	2011	Mukherjea et al. <sup>9</sup>
Nanoparticles	2011	Pyykko et al. <sup>10</sup>
Sensorineural hearing loss, animal model for evaluation	2012	Rivera et al. <sup>11</sup>
New technologies on drug delivery device	2012	Pararas et al. <sup>12</sup>

**Table 2** Comparison of various strategies in inner ear drug delivery.

Strategy	Efficiency	Safety
Systemic strategies	Low	Low safety, side effects caused by high systemic doses over time
Intratympanic strategies	Moderate, produce variable outcome	High safety, minimally invasive procedure that can be performed in a physician's office
Intracochlear strategies	High	Limited safety, precise surgery is needed and potential of serious complications

induce endothelial cell “shrinkage” and thereby lead to the opening of BLB tight junction<sup>23</sup>. Glycerol, an osmotic agent, was shown to increase the concentrations of drugs such as vasodilators and steroids in the lymph of the inner ear after systemic administration. In addition to chemicals, hearing disorders such as autoimmune inner ear disease (AIED), Meniere’s disease, meningitis-associated labyrinthitis, and genetic diseases can also affect the BLB.

## 2.2. Round window membrane

The round window membrane (RWM) is a soft tissue barrier separating the middle ear from the inner ear. It is the main passage for drug delivery from the middle ear cavity to the inner ear. The RWM is made up of an outer epithelial layer facing the middle ear cavity, middle connection layer, and inner cellular layer facing the scala tympani (ST) perilymph<sup>24–26</sup>. The variable RWM thickness and conditions of the membrane across the patient population is believed to be a factor leading to patient-to-patient variability in intratympanic inner ear drug delivery. Additionally, the pseudomembrane of the RWM (plugs of connective or adipose tissue) also accounts for this variability<sup>27</sup>.

The RWM acts like a semipermeable membrane. It is permeable primarily to low molecular weight molecules such as aminoglycoside antibiotics and corticosteroids. Large molecules, such as horseradish peroxidase (MW 45,000), can also diffuse through RWM under normal physiological conditions<sup>28</sup>. While larger molecules like albumin (MW 70,000) cannot diffuse across the RWM easily under normal conditions, such large molecules have been shown to penetrate the RWM into the inner ear during the early phase of inflammation<sup>29</sup>. Besides the molecular weights of the drugs and disease states of the ear, other factors including the integrity of the RWM and drug lipophilicities and charges also affect the rates at which the molecules diffuse across the RWM<sup>14</sup>. In addition, the permeability of RWM is affected by local treatments with anesthetics<sup>30</sup>, endotoxins and exotoxins<sup>31</sup>, histamine<sup>32</sup>, osmotic disturbances, and benzyl alcohol (a commonly used preservative)<sup>33</sup>.

## 3. Administration routes for inner ear drug delivery

### 3.1. Systemic route

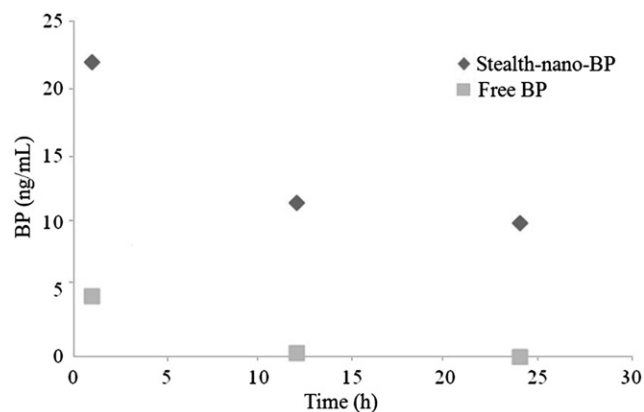
Generally, drugs are delivered to the inner ear *via* the systemic route, but only a few drugs can reach the target site of action at therapeutic concentrations in the inner ear because of the presence of BLB. In order to achieve therapeutic levels of drugs in the inner ear, high systemic doses are required, which are often associated with undesirable side effects<sup>1,4,34</sup>. Such systemic toxicities and side effects can range from minor nuisances to potentially life-threatening situations<sup>4</sup>. Despite these adverse effects, systemic delivery through oral, intravenous, and intramuscular routes is still considered as the most convenient method of drug administration to the inner ear and is currently accepted as the first line approach in the treatment of inner ear disorders. Systemic corticosteroids, for example, are used in the management of sudden sensorineural hearing loss (SSNHL) and AIED<sup>35,36</sup> in spite of potential side effects such as hypertension, irritability, cushingoid appearance and organ damage associated with long-

term systemic steroid therapy<sup>37</sup>. Several studies have demonstrated that systemic administration of lidocaine can relieve tinnitus, but the treatment of tinnitus by lidocaine systemically involves the risks of arrhythmia and central nervous system excitation or depression<sup>38</sup>. Another treatment of inner ear disorders *via* the systemic route is the use of streptomycin and gentamicin, which are ototoxic, in severe bilateral Meniere’s diseases<sup>39,40</sup>. The lack of selective pharmacological effects of systemic streptomycin and gentamicin on hearing and balance has led to hearing loss in clinical interventions of Meniere’s diseases<sup>41</sup>. Other drugs that have been delivered systemically to the inner ear in conjunction with gentamicin are glutathione<sup>42</sup>, salicylate<sup>43</sup>, alpha-tocopherol<sup>44,45</sup>, trimetazine<sup>46</sup> and flavonoids<sup>47</sup> for their protective effects on hearing loss or histological damages in gentamicin treatment.

Encouraged by the successful application of nanoparticles targeted to the brain, researchers have investigated the potentials of nanoparticles in inner ear drug delivery by systemic administration. Tamura et al.<sup>48</sup> found that the systemic application of poly lactic/glycolic acid (PLGA) nanoparticles of rhodamine provided targeted delivery of rhodamine to the liver but not the cochlea. Horie et al.<sup>49</sup> discussed the limited capability of nanoparticles for sustained and/or targeted delivery of drugs to cochlea after systemic application, which might be related to rapid clearance of the nanoparticles from circulation by the mononuclear phagocyte system (MPS) in the liver and spleen. They subsequently examined the efficacy of stealth nanoparticles encapsulating betamethasone phosphate (BP) for the treatment of noise-induced sensorineural hearing loss in mice. The results in their study demonstrated that stealth-nano-BP could deliver higher level of BP to the cochlea than free BP without the nanoparticles (Fig. 1). In addition to the pharmacokinetic data, immunohistochemistry for the glucocorticoid receptor showed remarkable enhancement of glucocorticoid receptor nuclear translocation in outer hair cells of cochlea treated with the stealth-nano-BP. This treatment provided functional and histological protection of the cochlea from the trauma of noise as compared to those with free BP. However, this method was still accompanied by a high drug concentration in the blood plasma as well as in organs such as the liver, which might lead to adverse systemic effects. With the problems in systemic drug delivery, there is a need to design a safer and more effective drug delivery system for the treatment of inner ear disorders.

### 3.2. Intratympanic route

In the last two decades, the topic of treating inner-ear disorders by local drug delivery has attracted considerable interest. Intratympanic delivery to the inner ear was performed *via* the injection or perfusion of the drug to the middle ear with the aim of drug diffusion through the RWM into the inner ear. This route of drug delivery was introduced more than half a century ago for the treatment of Meniere’s disease with local anesthetics<sup>50</sup> and antibiotics<sup>51,52</sup> and has been widely used in clinics since 1990s. This approach possesses several advantages over systemic drug delivery as this local drug delivery method can bypass the BLB, and therefore result in higher drug concentrations in the inner ear fluids and avoid undesired systemic exposure<sup>1,4,34</sup>. A number of clinical studies have been published on intratympanic injections of corticosteroids for Meniere’s diseases<sup>53–55</sup> and SSNHL<sup>56,57</sup>. Obstacles of intratympanic drug



**Figure 1** Betamethasone phosphate (BP) concentrations in the cochlea after systemic applications of stealth-nano-BP or free BP. The concentrations of BP in the cochlea after stealth-nano-BP application (diamonds) were significantly higher than those after free BP application (squares). The level of BP in the cochlea after stealth-nano-BP application was also maintained for a longer period of time. Data were obtained from Horie et al.<sup>49</sup>

delivery include the anatomic barriers to drug absorption from the middle ear to the inner ear such as the RWM, loss of drug in the middle ear through the Eustachian tube, and highly variable or unknown pharmacokinetic profiles of medications delivered *via* this route<sup>27,34,58</sup>. The percentage of drugs entering the inner ear following intratympanic injections can be relatively low. For example, one study showed that gentamicin reaching the basal turn was in the order of 2.5% of the applied drug after intratympanic injection<sup>59</sup>. Drug clearance in the cochlea is also a major factor that leads to a base–apex concentration gradient. Due to this concentration gradient, it may be difficult to treat hearing disorders in the middle and lower frequency ranges (disorders in the apex area) by the intratympanic drug delivery approaches. In addition, it is difficult to predict the amounts of drugs delivered to the cells in different turns of the cochlea even though computational simulations have been used to study the base–apex concentration gradient<sup>60–62</sup>.

Effective drug delivery to the inner ear *via* the intratympanic route also relies on the contact time of the drug solution (or drug delivery system) with the RWM. Unfortunately, large portions of the administered drugs are usually eliminated through the Eustachian tube following intratympanic drug delivery. There have been efforts to overcome this limitation through the development of devices and sustained-release drug delivery systems. This is the focus of the present review paper and is discussed in Section 4.

### 3.3. Intracochlear route

Like intratympanic delivery, the intracochlear delivery approach provides an alternative to systemic drug delivery to the inner ear. Direct intracochlear drug delivery can bypass the middle ear and allow drugs to get to their intended sites directly. Intracochlear delivery can substantially increase drug bioavailability in the inner ear and has the highest efficiency among the inner ear delivery methods discussed in this review. Numerous intracochlear delivery technologies are being developed to improve the efficiency of drug delivery to the inner ear. They include direct injections, cochlear implants, osmotic mini-pumps, as well as reciprocating perfusion

systems. Direct injection is the injection of drug solution directly into the cochlea through the RWM with a microsyringe and a narrow-gauge needle. This provides accurate drug delivery for acute drug application to the base of the cochlea. Cochlear implant is a device inserted into the ST in the treatment of inner ear disorders. The basic mechanism behind this treatment is to directly stimulate SGNs with electrical pulses through an electrode, overcoming the loss of hair cells in the cochlea<sup>5</sup>. However, it was recognized that the process of electrode insertion deep into the cochlea could destroy the remaining acoustic hearing due to various complications associated with implantation<sup>5</sup>. Thus, this method is most used for the treatment of severe to profound SSNHL. A recent development with cochlear implant was the concurrent use of drugs with implant to reduce trauma to the inner ear or to prevent further degeneration of hearing after implantation<sup>63,64</sup>. Osmotic mini-pumps are used to directly deliver drugs into ST *via* cannula, and reciprocating perfusion systems are similar to osmotic mini-pumps with the main difference that there is no accompanying net volume change of fluid in the reciprocating perfusion systems<sup>65</sup>. Recently, Sewell et al.<sup>66</sup> developed a implantable reciprocating inner ear drug delivery system which could provide time-sequence release of multiple agents for therapeutic applications. This approach is suggested to be best suited for controlled automatically complex dosing of numerous compounds. Although intracochlear strategies are more efficient than intratympanic delivery, they carry significant risks<sup>7</sup>. Based on observations in surgical procedures that involve perforation of the inner ear such as stapedectomy or the cochlear implantation on patients, it is well known that perforation and surgical manipulation of the ear leads to a significant risk of deafness<sup>67,68</sup>. Currently, a safe and robust technique for intracochlear delivery of drugs is not available.<sup>3</sup> These technologies have been summarized in a recent review paper<sup>7</sup> and will not be discussed here in detail.

## 4. Intratympanic approaches to inner ear drug delivery

### 4.1. Cannula-based delivery systems

Several cannula-based delivery systems are available commercially for sustained delivery of drugs to the middle ear. These devices include Silverstein Microwick and Round Window Microcatheter. Potential problems and adverse effects of these devices include the persistent perforation of the tympanic membrane, risk of infection in the middle ear or external ear, and tissue growth in the middle ear either in the form of fibrosis or epithelial ingrowth leading to cholesteatoma<sup>69,70</sup>.

#### 4.1.1. Silverstein Microwick

The Microwick (Micromedics, Inc., Eagan, MN) is a single-wick device placed in the round window niche through a tympanostomy opening<sup>71</sup>. The distal end of wick is in the external auditory canal, where the patients can instill medications several times a day for several weeks. Recent studies have demonstrated the reliability and consistency of gentamicin and methylprednisolone delivery by this device<sup>72</sup>. Long-term followed-up of 69 Meniere's disease patients treated with gentamicin three times per day using this system has demonstrated vertigo control in 53 patients (76.8%)<sup>73</sup>.

#### 4.1.2. Round Window Microcatheter

The Round Window Microcatheter is a double lumen-sustained microcatheter inserted into the round window niche designed to deliver drugs to the inner ear.<sup>74</sup> The catheter can be inserted into the middle ear and connected to a pump. This system has the advantage of delivering drugs to the middle ear continuously for several days to a few weeks. For example, by using a minipump attached to a microcatheter, gentamicin could be delivered for 10 days<sup>74</sup>. In another report, Panomat pump provided continuous delivery of glucocorticoid for a period of up to 4 weeks<sup>75</sup>. The microcatheter system has also been used for intratympanic infusion of steroids in patients with sensorineural hearing loss who have failed in the treatment with intravenous steroids and vasodilators<sup>76</sup>. When the microcatheter was used to deliver gentamicin to the RWM to treat the symptoms in Meniere's diseases, 60–83% patients got relief of their tinnitus and 89–100% patients recovered from vertigo symptoms, which is a significant improvement over the cure rates reported with conventional intratympanic administration of gentamicin<sup>77</sup>.

#### 4.2. Sustained-release systems

Sustained-release drug delivery systems can increase the residence time of a drug in the middle ear and provide controlled drug delivery to the inner ear. A number of systems including hydrogels and nanoparticles have been studied for this application (Table 3). These systems generally sustain drug delivery by the mechanisms of slow degradation of the material, slow drug diffusion, or a combination of both.

##### 4.2.1. Hydrogels

Gelfoam<sup>®</sup> is a biodegradable gelatin polymer that was first introduced in middle ear surgery and recently used as a drug delivery system for the inner ear. A recent study has demonstrated improved outcome of Meniere's disease treatment by the placement of Gelfoam<sup>®</sup> soaked in gentamicin on the RWM, which eliminated vertigo and tinnitus in 75% and 48% of the patients, respectively<sup>78</sup>. In another study, Havenith et al.<sup>79</sup> applied Gelfoam<sup>®</sup> infiltrated with brain-derived neurotrophic factor (BDNF) onto the RWM of deafened guinea pigs and evaluated the effect of this treatment by structural and functional measures. In the 2 weeks of Gelfoam<sup>®</sup> BDNF treatment, survival of spiral ganglion neurons (SGNs) in the inner ear was observed in the low turn of the cochlea, but no significant improvement was observed in the apical turn of the cochlea, probably due to the small amount of drug reaching the apical turn. This suggests that local delivery of BDNF using Gelfoam<sup>®</sup> can protect the SGNs in parts of the cochlea. However, Richardson et al.<sup>24</sup> did not achieve SGN survival after 4 weeks of treatment using Gelfoam<sup>®</sup> loaded with neurotrophin-3 (NT-3) on the RWM of deafened guinea pigs.

Several hydrogel systems for inner ear drug delivery employ hyaluronic-based chemistry. Hyaluronic acid (HA) is an anionic, nonsulfated glycosaminoglycan polysaccharide that is present in human body. It has been used as material in otology due to its well-known safety profile<sup>80</sup>. A commercial hydrogel Seprapak<sup>™</sup> (hyaluronic acid–carboxymethyl cellulose polymer, Genzyme Corporation) has been examined for the delivery of dexamethasone to the inner ear. The use of dexamethasone with

Seprapak<sup>™</sup> via the intratympanic route on the RWM was found to reduce both low and high frequency hearing loss associated with trauma of cochlear implantation<sup>81</sup>. Similarly, *n*-acetyl cysteine with Seprapak<sup>™</sup> was shown to be effective to protect the residual hearing after implant surgery in the high frequency hearing region near the point of implantation<sup>82</sup>. James et al.<sup>83</sup> investigated the concentration of dexamethasone in the cochlea after intratympanic delivery with Seprapak<sup>™</sup> and found significantly higher and sustained concentration of the drug in the cochlea than that by the drug alone without Seprapak<sup>™</sup>. These results were similar to those reported by Borden et al.<sup>84</sup> who utilized the thiol-modified HA gelatin hydrogel as a drug delivery system. Saber et al.<sup>80</sup> suggested that hyaluronic gel had no toxic effect on the hair cells but found that the gel temporary increased the thickness of RWM. The membrane returned to normal after 4 weeks, indicating that this material caused no permanent structural damage to the RWM.

Several other hydrogels were also studied for inner ear drug delivery through the intratympanic route. For example, a hydrogel of glutaraldehyde cross-linked with porcine type-I collagen was examined for the sustained delivery of BDNF to the inner ear. This treatment was shown to provide a protective effect manifested by stable auditory brainstem response (ABR) thresholds and preservation of spiral ganglion neuron densities in animals<sup>85</sup>. In another study, gelatin hydrogel loaded with hepatocyte growth factor was found to provide sustained drug delivery to the inner ear in guinea pigs<sup>86</sup>. Saber et al.<sup>87</sup> compared the feasibility of three structurally different chitosans as bioadhesives to deliver drugs to the inner ear through the RWM. All these bioadhesive gel formulations provided effective neomycin delivery to the cochlea over 7 days. The chitosans were free from any detectable toxicity on the cochlear tissue. Among the chitosans studied, glycosylated chitosan was considered as a promising biomaterial for inner ear therapy due to its safety and efficiency.

Another type of hydrogels for sustained drug delivery to the inner ear is temperature sensitive polymers (*e.g.*, sol–gel polymers). These polymers provide the advantage of solution to gel transition near body temperature. Particularly, the sol–gel polymer with a drug can be delivered as a solution through intratympanic injection using a narrow-gauge needle at room temperature, and the polymer solution then becomes a gel when it reaches the RWM in the middle ear at body temperature. Among these sol–gel polymers, poloxamer solution is liquid at room temperature and forms a gel after intratympanic injection, increasing the residence time of the drug in the middle ear. Salt et al.<sup>88</sup> evaluated the potential of a poloxamer hydrogel formulation containing dexamethasone in intratympanic delivery to the RWM of guinea pigs and analyzed the effects of the duration of drug application on drug concentration in the cochlea of guinea pigs and humans. Besides enhancing drug delivery to the inner ear, the increase in residence time of the drug on the RWM could also lead to more uniform drug distribution in the cochlea. Particularly, the drug concentration gradient along the length of the cochlea was shown to be significantly affected by the duration of drug application (*i.e.*, residence time on RWM): higher drug concentration and smaller drug concentration gradient in the cochlea were observed with prolonged drug delivery on the RWM (Fig. 2). In addition to the study by Salt et al.<sup>88</sup>, Wang et al.<sup>89</sup> also utilized poloxamer combined with micronized dexamethasone in inner ear delivery to prolong drug residence in the ear. Paulson et al.<sup>90</sup> developed a

**Table 3** Brief review of recent developments in intratympanic drug delivery.

Drug delivery system	Drug	Significant finding	Ref.
Gelfoam <sup>®</sup>	Gentamicin	Provided more consistent outcomes with transtympanic delivery of gentamicin, controlled vertigo in 75% of cases and improved tinnitus in 48% of the cases	78
Gelfoam <sup>®</sup>	Brain-derived neurotrophic factor (BDNF)	Local BDNF treatment enhanced the survival of cells in basal turn of the cochlea	79
Gelfoam <sup>®</sup>	Neurotrophins-3	No protective effect on the cells in the cochlea of guinea pigs	24
Hyaluronic gel	Neomycin	Had no toxic effect on the hair cells but temporary increased the thickness of RWM	80
Seprapak <sup>™</sup>	Dexamethasone	Provided protection of hearing across the entire frequency domain (2–32 kHz)	81
Seprapak <sup>™</sup>	<i>N</i> -acetyl cysteine	Increased the level of residual hearing at 24–32 kHz 4 weeks post-surgery compared to the controls	82
Seprapak <sup>™</sup>	Dexamethasone	Provided higher and sustained drug concentrations in the cochlear fluid	83
Thiol-modified Hyaluronic acid	Dexamethasone	Provided higher and sustained perilymph drug concentration	84
Glutaraldehyde cross linking of porcine type-collagen	Brain-derived neurotrophic factor	Spiral ganglion neuron (SGN) densities were greater than controls in the basal turn of the cochlea 3 months after implantation	85
Gelatin	Growth factor	Reduced the noise exposure-induced ABR threshold shifts and the loss of outer hair cells in the basal portion of cochlea	86
Chitosan glycosylated derivative	Neomycin	A safe and effective carrier for inner ear therapy although causes the round window membrane (RWM) to swell	87
Poloxamer 407	Dexamethasone	Provided more uniform distribution of drug in the inner ear	88
Poloxamer 407	Dexamethasone	Sustained drug levels in the inner ear for a period of at least 1–2 weeks	89
Chitosan glycerophosphate hydrogel	Dexamethasone	Provided measurable dexamethasone in perilymph for 5 days, and auditory testing revealed a temporary hearing loss, which resolved by the 10th post-operative day	90
Nanoparticles			
Lipid core nanocapsules poly L-lysine (HBPL) nanoparticles		Distributed throughout the human inner ear cell populations through the RWM of human temporal bone	91
Superparamagnetic iron oxide nanoparticles	Dextran	Distributed through a 3-cell layer RWM model under an external magnetic field	92
Superparamagnetic iron oxide nanoparticles encapsulated Pluronic F127 copolymer		Nanoparticles were seen throughout the inner ear cells	93
Polymersome		Provided specific targeting and binding affinity to SGNs, Schwann cells, and nerve fibers	94
Superparamagnetic iron oxide nanoparticles encapsulated PLGA nanoparticles		Provided nanoparticle delivery in RWM models ( <i>in vitro</i> cell culture, <i>in vivo</i> rat and guinea pig, and <i>in vitro</i> human temporal bone) under a magnetic field	95
PLGA nanoparticles	Rhodamine	Higher distribution of rhodamine in the cochlea	48
Silica nanoparticles		Cy3-labeled nanoparticles were found in the sensory hair cells and the SGNs	96
Lipid nanocapsules		Provided delivery to SGNs, organ of Corti and lateral wall with no hearing impairment, cell death, or morphological changes in the inner ear	97,98

Gelfoam<sup>®</sup>: gelatin sponge; Seprapak<sup>™</sup>: hyaluronic acid–carboxymethylcellulose polymer.

drug delivery system based on a chitosan glycerophosphate hydrogel, a biodegradable matrix that is temperature-sensitive to achieve sustained delivery of dexamethasone from the RWM over 5 days. The chitosan glycerophosphate hydrogel system was found to be safe, with no identified toxicities or complications from the procedure in a murine model. Ten days after the treatment, hearing thresholds returned to pretreatment baseline levels after an initial transient elevation of the threshold. This temporary hearing loss was suggested to be a result of conductive hearing loss related to the presence of the hydrogel in the middle ear and/or fluid associated with immediate post-operative changes.

#### 4.2.2. Nanoparticles

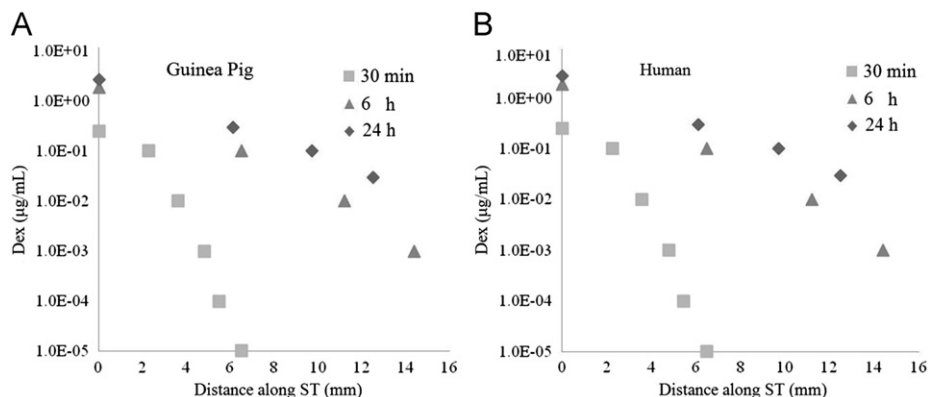
In addition to hydrogel systems, nanoparticles have been studied for drug delivery through the RWM to the inner ear. Because nanoparticles can offer targeted drug delivery to specific cells in the cochlea, they provide certain advantages over conventional drug delivery methods. Roy et al.<sup>91</sup> compared the ability of three types of nanoparticles to permeate the RWM on fresh frozen human temporal bone: polymersome nanoparticles of amphiphilic poly (ethylene glycol)-b-poly ( $\epsilon$ -caprolactone) (PEG-b-PCL) block copolymers, lipid core nanocapsules (LNCs) of lecithin and stearate of PEG, and nanoparticles of hyperbranched poly L-lysine (HBPL). It was found that these nanoparticles can pass through the RWM *in vitro*. Mondalek et al.<sup>92</sup> tested a delivery system of superparamagnetic iron oxide nanoparticles (SPIONs) through a 3-cell layer RWM model *in vitro*. The results showed that SPIONs distributed throughout the model membrane under an external magnetic field. In another study, Thaler et al.<sup>93</sup> investigated the capability of ferrogel consisting of SPIONs and Pluronic F127 with an imaging tag for the delivery of therapeutic agents across the RWM of cadaver human temporal bones as well as in organotypic explant cultures of mouse inner ears. It was found that the SPIONs were in the cytoplasm in organotypic explant culture, suggesting that the nanoparticle system can be a suitable cell specific drug delivery vehicle that prevents drug degradation in the cell endolysosomal compartment during drug delivery. Roy et al.<sup>94</sup> examined the cell targeting ability and toxicity of nerve growth factor-derived ligand functionalized polymersome nanoparticles for specific cell targeting to SGNs in mouse cochlear

organotypic culture and observed specific targeting to SGNs, Schwann cells and nerve fibers in the cochlear culture. However, due to the potential difference between RWM permeability *in vitro* and *in vivo*, the presence of cochlear clearance *in vivo*, and the differences between *in vitro* cell culture models and human cells *in vivo*, it is unclear if the *in vitro* permeation and cell culture results can be extrapolated to humans *in vivo*.

With the differences between *in vitro* and *in vivo* experiments, *in vivo* studies of inner ear drug delivery are preferred. Among the nanoparticle studies *in vivo*, superparamagnetic iron oxide encapsulated in PLGA nanoparticles were investigated and found to distribute throughout all turns of the cochlea of chinchilla<sup>95</sup>. Tamura et al.<sup>48</sup> showed higher distribution of rhodamine to the cochlea after application of rhodamine encapsulated PLGA nanoparticles on RWM as compared to systemic application, illustrating that PLGA nanoparticles can be a useful drug carrier for inner ear delivery. Besides PLGA-based nanoparticles, Praetorius and co-workers reported that silica nanoparticles labeled with fluorescent cyanine dye could be delivered to inner hair cells, vestibular hair cells, and the spiral ganglia by the application of the nanoparticle solution to the RWM<sup>96</sup>. These nanoparticles were observed not to alter hearing threshold, and no cytotoxicity was found to be associated with the treatment. Zou et al.<sup>97</sup> evaluated the ability of LNCs for inner ear drug delivery and found that these nanocapsules rapidly reached the spiral ganglion cells, nerve fibers, hair cells and spiral ligament fibrocytes after placement on the RWM. The biocompatibility of these nanocapsules in inner ear drug delivery was also evaluated. It was shown that the administration of nanocapsules did not cause hearing loss, cell death or morphological changes in the inner ear for up to 28 days after the application<sup>98</sup>. In summary, nanoparticles are a promising approach for inner ear drug delivery *via* intratympanic administration, especially for gene delivery because of their cellular uptake properties.

## 5. Biotechnology-based approaches of inner ear drug delivery

Biotechnology-based drug delivery has been used extensively in various fields of drug delivery. For inner ear delivery, biological therapies of inner ear disorders have been investigated to provide long-term drug delivery with the advantages



**Figure 2** Effect of the duration of dexamethasone (Dex) application to the round window of guinea pig (A) and human (B) upon the concentration gradient of Dex along the length of the cochlea. The data suggest an increase in the contact time of the drug delivery system with the RWM, *e.g.*, *via* sustained drug delivery systems, can provide a more uniform drug distribution in the cochlea. Symbols: 30 min application, squares; 6 h application, triangles; 24 h application, diamonds. Data were obtained from Salt et al.<sup>88</sup>.

of cell specificity, cell regeneration and/or cell replacement<sup>4,58</sup>. Biotechnology-based approaches to inner ear treatments include gene and stem cell therapies.

### 5.1. Gene therapy

Studies on the cellular and molecular mechanisms governing hair cell differentiation and regeneration in animal model systems have identified genes that may be targets of genetic manipulation in humans that can lead to protection, replacement, and/or regeneration of functional hair cells, supporting cells, spiral neurons and strial cells<sup>99</sup>. Until recently, systems for gene transfer to the inner ear have mainly focused on the utility of replication defective viral vectors, including adenovirus, adeno-associated virus (AAV), and herpes virus<sup>100</sup>. For example, adenoviruses were shown to transfer functional marker genes such as beta-galactosidase ( $\beta$  gal) and green fluorescent protein (GFP) as well as genes that alter the biology of the inner ear, such as glial-derived neurotrophic factor (GDNF), to the auditory system<sup>101,102</sup>. Besides viral vectors, liposomes have been investigated as a non-viral delivery system but were found to be less efficient in gene transfer than viral vectors<sup>100</sup>. Recently, Praetorius and co-workers<sup>96</sup> suggested that silica nanoparticles might be a potential non-viral vector for the sensory hair cells and spiral ganglion cells in the cochlea and the vestibular organ when the nanoparticles were administered to the round window niche. Other recent development includes the findings by Tan et al. of polycationic-mediated cochlear gene transfer with linear polyethylenimine *via* cochleostomy and osmotic pump infusion method<sup>103</sup> but the polyethylenimine has relatively low transfection efficiency as compared with viral vectors.

The identification of gene targeting for inner ear disorder and the demonstration of effective gene transfer for gene delivery have shown promise in gene therapy for inner ear disorders. However, successful cochlear gene delivery relies not only on the gene delivery systems but also on the delivery routes<sup>58</sup>. Different routes of administration to the cochlea have been investigated with various purposes, such as maximizing transduced efficacy, reducing cochlear toxicity, and/or preserving hearing function. Viral transgenes injected directly into the ST can provide reporter gene expression in SGNs, hair cells, and supporting cells in the organ of Corti<sup>104–106</sup>. Injection of gene transfer vehicles directly into SM can result in efficient transduction of hair cells and supporting cells<sup>107,108</sup>. On the other hand, the intratympanic route that requires gene delivery across the RWM by diffusion can preserve the cochlear integrity, but offer less efficient transduction in the inner ear cells<sup>62</sup>.

### 5.2. Stem cell therapy

Stem cell therapy to treat hearing loss has recently received attention due to its potential to replace and/or protect hair cells and SGNs after deafness. The feasibility of stem cell therapy in the treatment of inner ear disorders to replace damaged hair cells has been previously reported<sup>109</sup>. It was suggested that the implantation of embryonic stem cells, fetal dorsal root ganglion and otocyst cells in the inner ear could be used to restore damaged hair cells<sup>110,111</sup>. These findings for cell transplantation have shown an opportunity to repopulate

damaged sensory epithelia in humans that might be an alternative to gene therapy to reverse profound deafness. In spite of these successes, an effective delivery method for stem cells to the inner ear is needed.

## 6. Concluding observations and future trends

The prospect of efficacious inner ear drug delivery in the future lies within either the non-invasively administered sustained release systems *via* the intratympanic approach or systems in which drugs are administered *via* targeted and highly efficacious intracochlear routes. This is because systemic drug delivery can lead to systemic adverse effects. Although the use of systemic nanoparticles (*via* systemic delivery) to achieve greater drug distribution to the cochlea has shown promise, adverse effects from the drugs or nanoparticles in systemic circulation and/or other organs remain a main concern. While the intracochlear approach can deliver drugs directly to the inner ear, it involves high risks such as inner ear trauma and hearing loss. Intratympanic drug delivery can bypass the BLB, resulting in higher drug levels in the inner ear with less systemic drug exposure. This approach is relatively safe compared to intracochlear drug delivery. However, inner ear drug delivery *via* the intratympanic route relies on drug permeation through the RWM, the barrier between the middle ear and inner ear. In addition, intratympanic drug delivery to the inner ear can be significantly affect by the residence time of drugs in contact with RWM, which in turn can impact the clinical outcome.

The main problem encountered in intratympanic drug delivery is the variability in drug delivery and unpredictable bioavailability *via* this route, partly due to the variable residence time of drugs in the middle ear. To overcome this problem, various sustained drug release systems have been employed to increase the residence time of drugs in the middle ear and prolong drug contact with the RWM. This includes the use of polymers, hydrogels, and nanoparticles as the sustained release systems. Another problem in intratympanic drug delivery is related to the rapid clearance of drugs in the cochlea, resulting in low drug concentration in the apical section of the cochlea. Hence, it is more difficult to treat hearing loss of low frequencies *via* this route. Reports on inner ear drug delivery systems have suggested that nanoparticles loaded with drugs can be distributed broadly in the inner ear and enhance the delivery of drugs to the hair cells as well as spiral neuron ganglions. The results in these studies are encouraging and suggest that this approach can be the future of inner ear disorder treatment.

Gene delivery and stem cell transplantation are also promising approaches that can be used to treat inner ear diseases. While recent studies using virus vectors for gene delivery have shown no significant toxicity, this risk remains a potential problem in clinical application because the inner ear is delicate and is located near the brain. Thus, until a complete understanding of the safety profiles of virus vectors, non-virus vectors might be better suited in gene transfer in the ear for clinical use. In addition, there is a lack of effective methods to deliver these gene delivery systems and stem cells to the inner ear, which needs to be addressed.

In order to develop an effective intratympanic drug delivery system, an adequate evaluation method for inner ear drug



delivery is required. To date, researchers have employed both *in vitro* and *in vivo* methods to investigate drug delivery systems. For *in vitro* studies, generally, their purpose is to characterize the physical properties of the drug delivery systems before the *in vivo* animal studies. Consequently, the ability of *in vitro* studies to predict *in vivo* results through, *e.g.*, *in vitro/in vivo* correlation, is important. However, there are few reports comparing the *in vitro* and *in vivo* methods. Particularly, potential differences between the RWM permeability *in vitro* and *in vivo* and the lack of cochlear clearance *in vitro* can be significant factors in the prediction of perilymph pharmacokinetics. Future research and development of an effective evaluation method for inner ear drug delivery are needed. For *in vivo* studies, guinea pigs are commonly used as an animal model since the morphology of guinea pig inner ear is similar to that of human. However, the interpretation of the pharmacokinetic data in guinea pigs requires the consideration of the routes by which drugs entered the cochlea and the distance from the basal turn to the apical turn in guinea pigs compared to humans, which could influence drug distribution in the cochlea. Accurate measurements of drug levels in the inner ear fluids can also be complicated. Considering the small volume of cochlear fluid in guinea pigs (*i.e.*, less than 10  $\mu$ L), the determination of drug concentration at different locations in the cochlea (*e.g.*, different turns of the cochlea) can present a technical problem and be a major source of errors in advancing our understanding of perilymph pharmacokinetics. Thus, caution must be exercised in the analysis of the pharmacokinetic results in the literature and the generalization of the results from *in vitro* evaluation methods and *in vivo* animal models to humans.

Besides the lack of an established method in inner ear drug pharmacokinetics research, the toxicity and safety of new inner ear drug delivery systems are generally not well established. The inner ear is a complicated and subtle organ. Adverse effects and complications in inner ear drug delivery can lead to severe side effects such as hearing loss. Potential ototoxicity of novel drug delivery systems should be carefully examined. To be truly clinically useful, the drug delivery systems should be both effective and safe, short of any major risks, during inner ear disease treatment. These are the current major hurdles of effective drug delivery to the inner ear.

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