Biomaterials for non-invasive trans-tympanic drug delivery: requirements, recent advances and perspectives

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Various non-invasive delivery systems have recently been developed as an alternative to conventional injections. Local transdermal administration represents the most attractive method due to the low systemic side effects, excellent ease of administration, and persistent drug release. The tympanic membrane (TM), a major barrier between the outer and middle ear, has a similar structure of the stratum corneum compared to the surface of the skin. After several attempts, non-invasive trans-tympanic drug delivery has been regarded as a promising option in the treatment of middle and inner ear diseases. The round window membrane (RWM) was a possible non-invasive delivery approach from the middle to inner ear. The improved permeability of nanocarriers crossing the RWM is a current hotspot in therapeutics for inner ear diseases. In this review, we include the latest studies exploring non-invasive trans-tympanic delivery to treat middle and inner ear diseases. Both passive and active delivery systems are described. A summary of the benefits and disadvantages of various delivery systems in clinical practice and production procedures is introduced. Finally, future possible approaches for its effective application as a non-invasive middle and inner ear drug delivery system are characterised.

1. Introduction

Middle and inner ear diseases are common problems, especially in children. Acute otitis media (AOM) is defined as an infection of the middle ear and is the second most common problem among paediatric patients requiring medication. Approximately 80% of all children experience one episode of otitis media in their lifetime. Permanent hearing loss and balance disorders are complications when middle and inner ear diseases are left untreated.

Non-surgical treatments are applicable for most middle and inner ear diseases. The mainstays of current clinical practice are oral antibiotics for systemic delivery and intratympanic injection (or intracochlear injection) for local delivery. The outcomes of systemic drug delivery are unsatisfactory for both middle and inner ear diseases with unpredictable drug concentrations in the targeted positions. The drug delivery efficiency of systemic administration is limited in the middle ear cavity. Common invasive drug delivery methods for the local administrative treatment of middle and inner ear diseases include intratympanic injection and intracochlear injection. The pain experienced in intratympanic injection and the general anaesthesia required for intracochlear injection are difficult to tolerate, especially for paediatric patients. Nevertheless, the administration of medication several times a day to uncooperative toddlers is challenging. Drug delivery through the Eustachian tube has been considered using nasoendoscopy. With the need for general anaesthesia and doubts about its efficiency, reports of the Eustachian tube approach are sparse.

The definition of non-invasive trans-tympanic delivery is the administration of a drug across an intact tympanic membrane (TM) to the middle and inner ear. Direct topical drug delivery crossing the TM to the middle ear cavity could be a promising alternative, but it is hampered by the barriers of the middle and inner ear. Topical drug delivery improves local drug concentration, avoiding the side effects of systemic antibiotic delivery while improving clinical experience compared with an invasive operation (intratympanic injection or intracochlear injection). However, the delivery approach from the external auditory canal (EAC) to the middle ear cavity is obstructed by the existence of the TM (Fig. 1). The TM is impermeable to most medicines. The round window membrane (RWM) between the middle and inner ear has been regarded as a potential drug...
delivery approach from the middle ear to the inner ear. The blood–labyrinth barrier (BLB) is a semipermeable boundary between the vasculature and the inner ear fluids, obstructing systemic antibiotic delivery into the inner ear.

Due to the similarity between the TM and the epidermis of the skin, the recent development of transdermal drug delivery has been translated into an exploration of non-invasive trans-tympanic delivery. A growing number of studies have evaluated various non-invasive trans-tympanic delivery methods for the treatment of patients with an intact TM, focusing on improvement in permeability, advances in drug stability, the attainment of controllable release, and generalization for different types of drugs (hydrophilic and hydrophobic drugs). Non-invasive trans-tympanic delivery has the potential to achieve high intratympanic drug concentrations while avoiding the painful experience of intratympanic and intracochlear injection.

The design of non-invasive trans-tympanic delivery systems is a hotspot in the treatment of middle and inner ear diseases. We aim to cover the latest developments in the field. The central elements involved in the exploration of non-invasive trans-tympanic delivery systems are summarized in categories, including active delivery systems and passive delivery systems (Fig. 1). The application and function of individual components are reported in detail. The most up-to-date comparative analysis of non-invasive drug delivery systems is provided, with discussions about the advantages and disadvantages of individual elements. Moreover, future orientations of non-invasive trans-tympanic drug delivery are discussed.

2. Structure of the external, middle, and inner ear

The human ear can be divided functionally and structurally into three parts: the external ear, the middle ear, and the inner ear. The major functions of the ear are hearing and equilibrium sensing.

2.1. Structure of the external ear

The external ear is the outside part of the ear, and contains two parts: the auricle (pinna) and the EAC. The EAC, a slightly S-shaped tube, extends laterally from the pinna and ends medially at the TM (length approximately 25 mm, diameter...
around 7 mm, Fig. 2). The TM separates the external ear and the middle ear. The EAC is responsible for collecting sound waves and conveying the vibrations to the TM. The irregular shape of the EAC makes it difficult for topical drug delivery to the inner part of the EAC compared to regular skin.

2.2. Structure and barrier of the middle ear

The middle ear, namely the tympanic cavity, is an air-filled and membrane-lined space located medially between the TM, Eustachian tube, cochlea, and auditory nerve. The Eustachian tube is a bony and cartilaginous tube connecting the middle ear with the nasopharynx, which equalizes air pressure between the atmosphere and the middle ear. The middle ear contains ossicles (malleus, incus, and stapes) transmitting sound vibrations from the TM to the inner ear. There are three layers composing the TM: (i) the outer layer, a stratified squamous keratinized epithelium lining the outer surface of the body; (ii) the middle layer, a fibro-elastic connective tissue rich in collagen; and (iii) the inner layer, a cuboidal mucosal epithelium, which is one part of the mucous membrane of the tympanic cavity (Fig. 2). The thickness of the human TM ranges from 80 to 100 μm with an average area of around 64.3 mm². The primary functions of the TM include transmitting sound from the EAC to the ossicles and acting as a barrier to protect the middle ear from foreign substances and infective organisms. Foreign objects, including drugs and other materials administered to the middle ear, can be eliminated through the Eustachian tube. The middle ear is separated from...
the inner ear by the RWM and the oval window membrane (OWM). The existence of the TM prevents topical drug delivery directly into the middle ear. The keratin- and lipid-rich stratum corneum results in the impenetrability of TM to all except some tiny and moderately lipophilic molecules. The stratum corneum is composed of dead corneocytes and lipids organized in lamellar crystalline bilayers.

2.3. Structure and barriers of the inner ear

The inner ear has three main parts: the cochlea, semicircular canals (the labyrinth) and the vestibule.26 The cochlea is a spiral-shaped cavity in the bony labyrinth containing the organ of Corti, the sensory organ of hearing. The organ of Corti has sensory hair cells and supports the auditory sensory epithelia. The semicircular canals are three tubes obligated to regulate balance and sense the position of the head (Fig. 2). Located between the middle ear and the cochlea, the vestibule makes up the middle portion of the inner ear. Sound waves pass from the stapes through the vestibule to the cochlea. The inner ear has two openings into the middle ear, which are covered by membranes. The OWM is placed between the middle ear and the vestibule while the RWM separates the middle ear from the cochlear duct. The RWM is a semi-permeable membrane consisting of three layers: (i) a single cell layer outer epithelium; (ii) a connective tissue layer with fibroblasts, blood vessels and elastic fibres; and (iii) an inner layer of squamous cells (Fig. 2).27 Allowing fluid in the cochlea to move, the RWM ensures that the hair cells of the basilar membrane are stimulated. The average thickness of the human RWM is 70 μm with an area of about 2.2 mm². A drug intended for inner ear delivery from the TM is generally transmitted through the RWM into the fluid spaces of the inner ear.28

3. Middle and inner ear diseases

The most frequent middle ear disease is infection, namely otitis media. The causes of otitis media are multifactorial. Eustachian tube dysfunction, bacterial or viral infection of the middle ear, and nasal inflammation produced by allergic rhinitis or upper respiratory infection are contributing factors.29 The most suspected pathogenesis of otitis media is poor drainage or obstruction of the Eustachian tube, leading to the accumulation of fluid in the middle ear cavity. Infection of the fluid of the middle ear can be caused by upper respiratory tract infections (URTI) through the Eustachian tube. The TM has rarely been regarded as a passage for infection because its impermeability to most pathogens, including viruses and bacteria.

Inner ear diseases involve the membranous labyrinth and feature the triad of vertigo, sensorineural hearing loss (SNHL), and tinnitus. The aetiology of inner ear diseases consists of ototoxic medication, infection, and autoimmune responses.30 SNHL indicates damage to the hair cells in the cochlear or the auditory nerve. Labyrinthitis usually results from a viral infection of the auditory nerve or labyrinth, leading to vertigo. The RWM has often been used as an approach for the treatment of inner ear diseases, including cochlear implant surgery.28

3.1. Middle ear diseases with potential trans-tympanic treatment

Otitis media affects up to 50% of children in different countries.2,31 Otitis media can be classified as AOM (lasts less than 6 weeks) or chronic otitis media (COM, lasts more than 3 months).3 Based on its pathological features, otitis media includes otitis media with effusion (OME) and chronic suppurative otitis media (CSOM). Half of the cases of serious hearing loss worldwide are caused by otitis media.32,33

The traditional treatment for otitis media is systemic antibiotics for mild cases, and invasive installation of pressure equalization tubes into the TM for patients with recurrent otitis media.34,35 Patients with recalcitrant otitis media had to choose surgical treatment after the failure of medical therapy.36

3.1.1. Acute otitis media. AOM is defined as an infection of the middle ear space. AOM, the second most prevalent paediatric diagnosis in an emergency department following URTI, is strongly associated with URTI involving the nasopharynx. Approximately 75% of children have at least one episode of AOM by school age.37 The complications of AOM include life-threatening meningitis and persistent hearing loss.38 Oral antibiotics are the mainstay of the treatment of uncomplicated AOM. Amoxicillin is the most frequently applied antibiotic with a duration of therapy of between 5 and 10 days for AOM patients.34

3.1.2. Chronic suppurative otitis media. CSOM involves a perforated TM with persistent drainage from the middle ear for more than 12 weeks. CSOM can result from AOM and Eustachian tube obstruction.39 The global prevalence rates of CSOM range between 1% and 46% with polymicrobial aetiology. Topical quinolone drops are the treatment for CSOM in consideration of their low ototoxicity. Quinolones are effective at resolving otorrhoea and eliminating microorganisms. Antibiotics can be used alone or in addition to steroids, improving their anti-inflammatory effect.40 Mastoidectomy and tympanoplasty are required in patients with recalcitrant symptoms after unsuccessful pharmacotherapy.41

3.2. Inner ear diseases with potential trans-tympanic treatment

There are various inner ear diseases, including Ménière’s disease, benign paroxysmal positional vertigo, and labyrinthitis. The hearing portion (cochlea) and the balance portion (semicircular canal) can both be affected.42 The treatment for inner ear diseases can alleviate the symptoms. Oral medicines (usually meclizine or Dramamine) are prescribed to control vertigo.43 Diuretics are applied to restrict the overproduction of fluid in the inner ear. The intratympanic injection of dexamethasone treats inner ear disease by reducing the swelling in cochlea hair cells. Surgical intervention is rarely offered for inner ear diseases.44

3.2.1. Sensorineural hearing loss. SNHL is a type of hearing loss caused by damage to the auditory nerve or the hair cells of
the inner ear. The overall prevalence of SNHL is 6.1% worldwide. The causes of SNHL are diverse: aging, autoimmune inner ear disease, cochlear otosclerosis, benign tumours and sudden hearing loss.

The treatment of SNHL depends on the aetiology of individual patients. Corticosteroids (oral or intratympanic injection) are used to reduce cochlear hair cell swelling and inflammation for cases such as sudden hearing loss. For severe SNHL, assistive listening devices or surgical treatment are applied.

3.2.2. Ménière's disease. Ménière's disease is an inner ear condition characterized by vertigo, tinnitus, and hearing loss. The prevalence and incidence of Ménière's disease vary depending on ethnic and geographic background (ranging from 3.5 per 100,000 to 513 per 100,000). Vertigo has a huge impact on normal daily activities. Permanent hearing loss may present in patients with Ménière's disease.

Diuretics are the most prescribed medications for Ménière's disease, restricting the overproduction of fluid in the inner ear. Oral meclizine is applied to control vertigo. For severe Ménière's disease, an intracochlear injection of dexamethasone is used to control vertigo. However, the effect of dexamethasone is not permanent.

4. Current non-surgical therapeutic techniques for middle and inner ear diseases

Surgical treatment is applicable for certain patients with middle and inner ear diseases of specific aetiologies. However, for most patients with mild to moderate disease, non-surgical treatment is effective at alleviating symptoms. The common symptoms of middle and inner ear can take a major toll on the daily activities of patients. The key goal of non-surgical treatment is to reduce the symptoms (vertigo and hearing loss) in patients with middle and inner ear diseases. Systemic drug delivery (oral medication) and local drug delivery (intratympanic administration and intracochlear administration) are important methods of non-surgical treatment for middle and inner ear diseases.

4.1. Systemic medication

The middle and inner ear are two anatomic structures located deep within the temporal bone. The TM separates the middle ear cavity from the EAC, making direct topical medication impossible to the middle and inner ear. The Eustachian tube is a passageway between the middle ear and the nasopharynx, and is the only possible approach for direct topical medication through the nose. However general anaesthesia is required for intranasal delivery to the Eustachian tube due to the strong discomfort caused.

Resulting from the limitations of direct topical delivery, conventional oral medication was introduced for middle and inner ear diseases. The oral medication was designed to reach the targeted organ through blood circulation after absorption into the bloodstream of the stomach and intestine. There were a variety of oral medications involved in the treatment of middle and inner ear diseases. Oral antibiotics (amoxicillin–clavulanate) were the first-line therapy for AOM. The most beneficial treatment for sudden hearing loss was corticosteroid administered over 1 to 2 weeks. Diuretics were the most prescribed oral medications for Ménière's disease to restrict the overproduction of fluid in the inner ear.

However, the application of systemic medication has an increased risk of systemic adverse reaction. Systemic antibiotics resulted in the amplification of antimicrobial resistance for common diseases. The intended targets (the middle ear and inner ear) are two relatively tiny portions of the body. The increased blood concentration of antibiotics or corticosteroids to guarantee sufficient drug concentration in the intended portion (the middle or inner ear) has the potential for raising the risk of systemic side effects. Adverse effects of corticosteroids include diabetes, hypertension, and hyperglycaemia. Additionally, the BLB between the vasculature and fluids of the inner ear restricts the entry of most blood-borne compounds into the inner ear.

4.2. Invasive local delivery

Considering the adverse effects of systemic drug delivery and the existence of the BLB, invasive local delivery methods (intratympanic injection and intracochlear injection) were introduced in the treatment of middle and inner ear diseases. Compared with non-invasive procedures, invasive medical procedures are defined as diagnostic and therapeutic methods by cutting or puncturing the skin or by inserting instruments into the body.

Intratympanic injection means the administration of a medication directly into the middle ear space through the TM. The operation of intratympanic injection uses a syringe needle to penetrate the TM to inject the medication into the middle ear. Intratympanic steroid injection is used to treat cochleovestibular symptoms of inner ear disease, including Ménière's disease and idiopathic sudden SNHL. Nevertheless, the pain and dizziness at the time of the injection are agonizing experiences in patients receiving intratympanic injection. Sclerosis or tympanosclerosis can occur after the installation of a ventilation tube. Secondary infection and persistent TM perforation are possible complications of intratympanic injection.

Intracochlear injection is defined as injection into the inner ear through the RWM, and is a reliable method for delivering medical substances to the cochlea. The operation of intracochlear injection opens the RWM with a sharp needle followed by the slow manual insertion of the prepared inner ear catheter. After administration, the catheter is removed, and the RWM is sealed with additional tissue. The goal of intracochlear injection through the RWM is to deliver medication in the treatment of inner ear diseases, including severe hearing loss, tinnitus, and vestibular balance disorders. Intracochlear administration through an opening in the cochlear bony wall is more efficient than intratympanic administration but rather
invasive. Intracochlear injection can be performed only during surgery in current clinical practice.

5. Potential requirements of non-invasive trans-tympanic drug delivery

Considering the limited efficiency of systemic drug delivery and the discomfort of intratympanic injection, non-invasive trans-tympanic drug delivery is a promising alternative in the treatment of middle and inner ear diseases. Non-invasive trans-tympanic delivery is a local administration method avoiding the side effects of systemic delivery and the pain of injection. Drug concentration in the targeted tissue should be more convenient to control in localized delivery.64 However, the clinical practice of non-invasive trans-tympanic delivery faces multi-dimensional challenges (Fig. 3).

5.1. Physical status of delivery systems in the EAC and contact with the TM

To achieve non-invasive trans-tympanic delivery across the TM, the delivery system should reach the outside of the TM through the EAC as the first step. The EAC is an irregular space (about 25 mm in length and 7 mm in diameter) beyond the reach of most delivery devices. The most common approach is for fluid to pass the narrow EAC and make contact with the TM.65 However, the lumen of the EAC for loading the liquid formulation is relatively small, and the liquid formulation inside the TM can easily flow away in an anatomical position. As a result, traditional ototopical drops require high administration frequency leading to inconvenience, especially for paediatric patients.

Hydrogels and solutions are possible physical forms for delivery into the deep end of the EAC. Adhesive and ductile liquid can assist contact between the drug carriers and the TM (Fig. 3). Gelation of liquid formulation on the outer surface of TM is a potential medium for controllable long-term delivery avoiding frequent administration.66

5.2. Potential mechanisms of non-invasive delivery systems crossing the TM and RWM

The targets of non-invasive trans-tympanic drug delivery are the middle and inner ear. In non-invasive trans-tympanic drug delivery, the drugs must cross the intact TM and RWM before diffusing into the middle and inner ear through the approach. Similar to transdermal delivery, the restriction of trans-tympanic delivery was attributed to the high impermeability of the stratum corneum layer in the TM (Fig. 3).12 The permeation of a drug through the intact TM is mostly equivalent to the process of transcutaneous delivery.67

The lipid-rich keratinized stratum corneum provides a barrier preventing the permeation of compounds from the EAC to the middle ear.68 The permeation of a drug through skin can occur through different approaches: (i) the intercellular pathway (the lipid bilayers occupying the intercellular spaces of the keratinocytes); (ii) the intracellular pathway (across the corneocytes), or (iii) the transappendageal pathway (hair follicles or sebaceous and/or sweat ducts).69 The most common penetrating approach is the intercellular path. Several permeation enhancement techniques have been utilized to affect the molecular architecture of the intercellular path.17,70 The factors associated with favourable percutaneous permeation include low molecular weight and moderately hydrophobic molecules.71–74

Fig. 3 Key design principles of improved non-invasive trans-tympanic drug delivery systems.
The semi-permeable RWM is a barrier between the middle and inner ear. The RWM consists of three layers with the tight junctions of the outer layer limiting the passage of most molecules. Insufficient drug transfer indicates the selective nature of substance entry from the RWM to the cochlear. Factors such as molecule size, configuration, concentration, liposolubility, electrical charge, and the thickness of the membrane influence the permeability of the RWM. The impermeability of the RWM is lower than that of the TM because of the absence of a lipid-rich stratum corneum in the RWM.14

5.2.1. Recent advantages of transdermal drug delivery systems. Various non-invasive drug delivery methods have emerged as alternatives to conventional invasive injections. A transdermal drug delivery system (TDDS) represents the most attractive method with regard to low rejection rate, convenience of administration, and persistence in patients.75 The physicochemical properties of the skin contain multiple obstacles and restrictions to transdermal delivery. Recent efforts have been made to explore different types of TDDS methods. The most important skin barrier effect of the epidermis occurs in the stratum corneum, the outermost layer. The biggest issue of TDDS is to resolve the barrier effect of the stratum corneum, deliver the drug to the skin tissue, and pass through the cellular and vascular tissue to arrive at the target tissue.76 Only a small number of substances can be delivered directly into the skin tissue.

The major categories of transdermal drug delivery include passive and active transdermal delivery.77 Passive drug delivery is based on the natural properties of drug carriers and the microenvironment while active drug delivery introduces external interventions to enhance specificity and control drug release at the target site. Enhancement in transdermal delivery by equipment (active delivery) introduced iontophoresis, sonophoresis, electroportation, photomechanical waves, and microneedles. Passive TDDS employs vesicles, polymeric nanocarriers, and chemical permeation enhancers (CPEs).78

5.2.2. Potential mechanisms of non-invasive trans-tympanic drug delivery. Drug delivery systems are applied to the TM before diffusing into the middle ear in non-invasive trans-tympanic delivery. Drug flux across barriers has been best studied in the field of transdermal delivery.75 The permeation of a drug through the skin is possible through the lipid bilayers (intercellular path), across the corneocytes (intracellular path), or through hair follicles (transcellular path). The most commonly employed method was the intercellular path with various of permeation enhancement techniques being explored.79 The TM is impermeable to all except specific small and moderately hydrophobic molecules. The consideration of permeability was more difficult for the TM than for the RWM.

The aims of a drug delivery system are to sustain the highest possible concentration outside the barrier for the longest possible period, to use drugs with the greatest permeability, and to apply the system over the greatest possible area. Passive dermal delivery has employed CPEs promoting the flux of a drug across barriers with potential in adaption of the TM. The recent developments of chemical enhancers and vesicles provide improvement for non-invasive trans-tympanic drug delivery.90 External stimuli (active delivery) may be effective approaches for non-invasive therapeutics of middle and inner ear diseases.

5.3. Pharmacokinetics of drugs in the middle and inner ear

Limited understanding of middle and inner ear pharmacokinetics is one of the challenges of trans-tympanic drug delivery. Studying inner ear pharmacokinetics in humans is hindered by the inability to sample inner ear fluids without invasive surgery. Small animals (such as guinea pigs) are frequently used for studies on middle and inner ear pharmacokinetics due to the resemblance between humans and specific animals.81

The pharmacokinetics of drug solutions were analysed in trans-tympanic delivery.82 Therapeutic monoclonal antibodies have been involved in the treatment of SNHL. The evaluation of pharmacokinetics by Kita et al. demonstrated the rapid transfer of monoclonal antibodies from the middle ear to the cochlear fluid in a guinea pig model.83 High permeability of the RWM to monoclonal antibodies was observed. The middle and inner ear pharmacokinetics of intratympanic injected dexamethasone were reported by Moatti et al. in a pig model.83 There was significantly more dexamethasone content than dexamethasone sodium phosphate (DSP) in the inner ear after solely DSP injection. Dexamethasone was cleared rapidly from the middle and inner ear (1–2 weeks).84 The pharmacokinetics of trans-tympanic free drug solutions demonstrated rapid drug clearance and an unstable form in the middle and inner ear.

Compared to free drug solutions, nanocarriers increase drug concentration and drug persistence in the middle and inner ear.85–87 The application of nanocarriers makes the drug entrance dependent on the characteristics of the nanocarrier rather than the drug. Nanoparticles can increase the concentration of hydrophilic drugs in the perilymph compared to the free drug solution.86 Cationic nanocarriers may be efficient at delivering non-degraded macromolecules to the inner ear.88

Additionally, nanocarriers can assist a prodrug to be converted into the active form. One of common corticosteroid, dexamethasone phosphate, must be converted into dexamethasone to be active. The introduction of nanocarriers seems to improve the drug pharmacokinetic profile in terms of degrees of drug persistence and concentration in the middle and inner ear.89 The nanocarrier can favour the drug diffusing through the middle ear barriers. The drug concentration in the perilymph and inner ear tissue can be increased as well. However, studies of the influence of nanocarrier degradation in the middle ear barriers are sparse.

6. Recent advances in the evaluation methodology of middle and inner ear delivery

Studying middle and inner ear pharmacokinetics in humans is hindered by the difficulties of sampling middle and inner ear fluids without surgery. Animal models are problematic because
of the mismatch of anatomy, histology, and size of the middle and inner ear between humans and animals. However, without an available evaluation system and an effective animal model, delivery devices requiring further development lack evaluation methods. There is a need for measures enabling high-throughput initial screening for trans-tympanic drug permeation.

6.1. Animal model of pharmacokinetics and ototoxicity in the middle and inner ear

The development of a translatable large animal model for assessing trans-tympanic delivery could be an opportunity to improve the drug delivery of new therapeutics. However, differences in inner ear dimensions and anatomical features complicate the transfer of experimental results to the clinic. The gap between rodents and humans may be bridged using larger animal models such as non-human primates. The application of non-human primates is challenging due to financial hurdles and operational complexity. Guinea pigs and piglets are potential representative animal models in studies of ototoxicity and hearing function after intratympanic drug delivery. The resemblance between humans and small rodents has contributed to the exploration of trans-tympanic drug delivery.

However, most animal models are limited by the finite number of temporal sampling points. There is a need for a physiologically relevant \textit{ex vivo} TM and RWM model to easily test and explore innovative delivery methods to the middle and inner ear across multiple time points.

6.2. Delivery efficiency assessment of drugs crossing the TM and RWM

Several TM models have used TM tissue from guinea pigs to reconstruct two cavities resembling the delivery chamber and receptor chamber. The TM of the rodent was five to six times thinner than the human TM, leading to failure of the direct translation of outcomes from the models. Additionally, the layered structure of human TM and RWM should be imitated in the explorative model, making the results of permeability more viable for humans.

Considering the above, Veit \textit{et al.} have designed and described an \textit{in vitro} three-dimensional (3D) model for the characterization of drug permeability across the TM using a physiologically based approach. The outer layer of the TM was mimicked through an \textit{in vitro} reconstructed epidermis due to the similarity with the skin. For the middle layer, human fibroblasts embedded in a collagen matrix were utilized to model the lamina propria of the TM (Fig. 4). The exploration included the effect of growth time on tissue morphology, tight junction formation, and drug permeation. The permeability of four sample drugs was presented to test the \textit{in vitro} model. The results suggest the outermost portion to be the most significant barrier to drug permeation, and most drugs underwent paracellular permeation in the outer layer. There appears to be a general trend of increased drug lipophilicity leading to higher permeability. Additionally, increased transcellular permeation, rather than paracellular permeation, caused an improvement in drug permeability. Based on the 3D model by Veit \textit{et al.}, further studies can be developed to mimic common TM diseases, to evaluate drug-induced damage and drug interactions related to TM integrity. Additionally, an \textit{ex vivo} porcine RWM model was developed by Moatti \textit{et al.} The designed RWM model was similar in structure and thickness to the human RWM with viability for five days. Drug passage could be evaluated at multiple timepoints. The \textit{ex vivo} TM or RWM model provided straightforward approaches to evaluate the
efficiency of non-invasive delivery methods to the middle and inner ear.

7. Potential components of non-invasive trans-tympanic drug delivery systems

The low permeability of the TM stratum corneum layer makes non-invasive delivery through an intact TM difficult. For the treatment of middle and inner ear diseases, an advanced drug delivery system has been modulated to permeate the TM with numerous drug-loaded nanocarriers included. However, most of the explored delivery systems were invasive and unrepeateable. Non-invasive long-term repeatable delivery effects showed a promising orientation for the future. Composite hydrogels were introduced to adapt the irregular lumen of the EAC. In situ gels were expected to adhere to the intact TM, increasing the effective delivery area. For an improvement in permeability and stability, different types of nanocarriers were explored in trans-tympanic delivery systems. Small molecules assisting the penetration process were considered.

Studies eligible for inclusion in the literature review of non-invasive trans-tympanic drug delivery must meet the following criteria: (i) original research identifying components permeating the TM or RWM with potential to develop non-invasive trans-tympanic drug delivery systems (not invasive methods, such as intratympanic injection or intracochlear injection); (ii) ex vivo or in vivo experiments must form part of the studies to evaluate the characteristics of the trans-tympanic delivery (such as delivery efficiency, permeability, biocompatibility, and toxicity); (iii) only articles published in English from Embase, MEDLINE, Web of Science Core Collection, and Google Scholar are included; and (iv) only articles published in the last decade. Finally, twenty-one original articles were selected within this scope (Table 1).

Table 1 Summary of studies focusing on non-invasive trans-tympanic delivery

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<td>Poloxamer 407 with Carbopol-940 and hydroxypropyl methyl cellulose</td>
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<td>Peptides</td>
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the skin and TM. The stability of the loaded drug can potentially be improved through the application of nanoparticles. Quantities of nanoparticles were developed to treat middle and inner ear diseases through intratympanic or intracochlear injection.\textsuperscript{85,115} For example, silk fibroin nanocarriers have been applied as biomaterials in various biomedical fields due to their excellent mechanical properties and exceptional biocompatibility.\textsuperscript{116} Intratympanic injection of natural multifunctional silk microcarriers has been reported for the treatment of noise-induced hearing loss in guinea pigs by Zhang hui et al.\textsuperscript{117} However, a limited number of nanocarriers were evaluated in non-invasive trans-tympanic drug delivery compared to invasive intratympanic and intracochlear injection.\textsuperscript{94} Excellent penetrability was regarded as a major property of novel nanocarriers required for non-invasive middle and inner ear delivery.

7.1.1. Nano-spanlastic vesicles. Nano-spanlastic vesicles are surfactant-based elastic nanovesicles, consisting of a non-ionic surfactant and an edge activator (EA).\textsuperscript{118} Nano-spanlastic vesicles are safe, biodegradable, and non-immunogenic vesicular carriers with low toxicity. Advantageous elasticity and chemical stability of nano-spanlastic vesicles contributed to increased deformability and permeability across biological membranes by enabling the vesicles to deform and squeeze through the epithelial layers. The potential of elastic carriers enhancing drug flux across the TM was evaluated by Al-Mahallawi et al.\textsuperscript{11} Span 60 based nano-elastic vesicles, nano-spanlastics with encapsulated ciprofloxacin for improved trans-tympanic delivery, were investigated (Fig. 5).\textsuperscript{11} Ciprofloxacin-loaded nano-spanlastics were prepared by a thin film hydration technique, using several non-ionic EAs based on a full factorial design. An evaluation of encapsulation efficiency percent (EE\%) reported that 20% was the optimum percentage for drug entrapment. Increasing the EA content beyond the optimum percentage led to a decrease in the EE\%. EA type (X\textsubscript{1}) significantly influenced the particle size (PS) of the prepared nano-spanlastics. The smallest PS was obtained with Brij 35. Brij 35 has the least bulky structure compared to other EAs. The PS of the prepared vesicles decreased upon increasing the EA content. The vesicle elasticity increased with rising EA level. Additionally, EA type (X\textsubscript{1}) and percentage (X\textsubscript{2}) revealed significant effects on the deformability index (DI) of the vesicles. The Brij 35-based formulae demonstrated the highest mean DI. The aim of the formula design was to maximize EE\% and DI and minimize PS, with Formula S-2 containing 20% Brij 35 with the highest desirability value.

An assessment of the selected formulation was conducted through \textit{ex vivo} permeation studies of rabbit TM. The encapsulation of ciprofloxacin into non-ionic surfactant-based vesicles (S-2) or nano-transferosomes resulted in an obvious
improvement in trans-tympanic drug delivery. The nanotransfersomes demonstrated better ex vivo trans-tympanic permeation comparing nano-spanlastic vesicles. The lower permeability parameters of nano-spanlastics can be interpreted by the degree of flexibility of both types of vesicle. The results demonstrated nano-spanlastics as a potential trans-tympanic delivery carrier for ciprofloxacin, the only drug tested in the study. Nevertheless, physical and chemical instability are major disadvantages of nano-spanlastic vesicles.

7.1.2. Liposomes. Liposomes are defined as sphere-shaped vesicles composed of one or more phospholipid bilayers. Great biocompatibility and ability to incorporate both hydrophilic and hydrophobic therapeutic agents make liposomes potential drug delivery carriers. The similarity between the lipid composition of liposomes and the epidermis enable liposomes to penetrate the epidermal barrier. The easy preparation of liposomes demonstrates most the affordable options among nanocarriers. The application of liposomes can improve the intercellular pathway. A novel pneumococcal endolysin (MSlys) with encapsulation in liposomes was characterized. The trans-tympanic permeation ability of liposomes loaded with pneumococcal endolysin MSlys was recorded ex vivo by Silva et al. Liposomes composed of α-alpha-lecithin and sodium cholate or PEG2000 PE loaded with MSlys endolysin were characterized. Loading an increased concentration of MSlys had no significant impact on the size and polydispersity of liposomes. The encapsulation efficiency of MSlys in L:SC and L:PEG liposomes was similar. PEG may act as a permeation enhancer. Compared with the free form, an increased quantity of MSlys was able to permeate sheep TM when using PEGylated liposomes (28%) after 24 hours. The results of human TM were consistent with the assessment of sheep TM. The enhanced drug penetration may be a result of the interaction of the liposome components with the stratum corneum lipids and subsequent fluidification. Loading of the pneumococcal endolysin MSlys in PEGylated liposomes enhanced its permeability across the TM. Endolysin-loaded PEGylated liposomes seem to be a promising approach for the trans-tympanic treatment of otitis media.

However, antipneumococcal activity was not observed 4 hours after permeation, with detection of hydrolysis of endolysin after an extended incubation time (more than 48 hours). The passive leakage of small molecules across the membranes is a major limitation of liposomal drug delivery. The phospholipid can experience oxidation and hydrolysis-like reactions, making the system unstable. The application of liposome drug delivery systems has been limited by short half-lives and low solubility. Liposomes can impart toxicity to normal tissue and evoke an immune response while being used to reduce systemic toxicity from the encapsulated agents. Current manufacturing processes of liposomes are generally complex multi-batch processes, while liposome preparation processes adopted in the laboratory setting are hard to transform into large-scale production.

Liposome formulations can be broadly divided into cationic, anionic, and neutral subtypes. Charged phospholipids are employed to formulate liposomes with different surface charges enhancing the permeation of active ingredients through epidermal layers. Multiple mechanisms are implicit in the cross-membrane transport of cationic liposomes, including phagocytosis, micropinocytosis, endocytosis, direct diffusion, and adhesive interactions. Cationic liposomes interact with negatively charged glycoproteins on the cell surface, increasing the affinity and residence time with the target cells to achieve improved drug delivery. Considering the specific requirements of non-invasive trans-tympanic delivery, cationic liposomes can be optimized to amplify efficacy in delivering encapsulated drugs. Interaction with cell membranes and improved muco-adhesion can help the penetration of cationic liposomes crossing the TM. The biodistribution and ototoxicity of cationic liposomal ceftriaxone (CFX) for non-invasive trans-tympanic delivery was reported by Shafiee et al. Cationic liposomes with a size of ~ 100 nm and a surface charge of +20 mV were employed to encapsulate CFX. In a chinchilla model of otitis media, the biodistribution was evaluated by confocal microscopy, while ototoxicity was evaluated by auditory brainstorm response and cochlear histology. Compared with free CFX, liposomal ceftriaxone (CFX-Lipo) displayed a 658-fold increase in drug delivery efficiency crossing the TM. No ototoxicity or systemic side effects were observed in chinchillas with applied CFX-Lipo. The cationic liposome designed for non-invasive trans-tympanic delivery could be a promising approach for the treatment of otitis media. Nevertheless, the stability and drug release rate of liposomes require further optimization to acquire long-term drug release. Cationic liposomes have the potential to activate pro-apoptotic and pro-inflammatory cascades, triggering a host immunogenic response, especially for macrophages.

7.1.3. Nano-transfersomal vesicles. Transferosomes are a special subclass of liposomes consisting of at least one inner aqueous compartment enclosed by lipid vesicles. The superior flexibility and less rigid structure drive transferosomes to penetrate deeper into the skin compared with liposomes. Al-Mahallawi et al. developed nano-transfersomal vesicles loaded with ciprofloxacin for the enhancement of the trans-tympanic permeation of an antibiotic. Sadabad and colleagues designed a trans-tympanic drug delivery mechanism for middle and inner ear treatments using transfersomes called liposomal nanoparticles (TLipo) (Fig. 6). The liposomal nanoparticles employed were vesicular structures composed of phospholipids arranged in a shell-like bilayer with a hydrophilic surface and a hydrophobic interior. Liposomal nanoparticles can fuse through lipid-rich barriers with great biocompatibility and biodegradability. TLipo equipped with CPEs can be one-tenth smaller in diameter in lipid-rich biological barriers through the channels, synthesized through a lipid film hydration method and labelled with a rhodamine-B (RhB)-based fluorescent dye to confirm their accumulation in the middle and inner ear. The size of the vesicles was verified with their shape and stability (Fig. 7). The TLipo vesicles were administered as ear drops into the ears of healthy mice, applied to the EAC and found to pass through the TM 3 hours after
administration, and confirming the presence of TLipo in smaller amounts in the inner ear. A higher accumulation of the vesicles in the middle ear was observed over time. The fluorescent TLipo-RhB in the middle ear started decaying after 1 week and disappeared after 1 month. Immunohistochemistry results demonstrated no evidence of hair cell loss in the cochlea 1 month after administration. However, the major limitation of transferosomes is the difficulty of loading hydrophobic drugs without compromising the deformability and elastic properties. The disadvantages of transferosomes were their relatively high price and oxidative degradation. For TLipo to be verified as a viable solution, it should be extensively tested before clinical application. The methods of therapeutic drug encapsulation for topical treatment require further study.

Fig. 6 Concept for non-invasive delivery to the middle ear using TLipo vesicles: eardrop carries TLipo vesicles penetrates the tympanic membrane without damaging it due to their similar lipid structure. Copyright 2022, American Chemical Society.

Fig. 7 Size of TLipo vesicles: (a) cryo-TEM images, (b) vesicle size distribution of TLipo vesicles before and after freeze-drying and encapsulating with RhB fluorescent dye. Copyright 2022, American Chemical Society.
7.1.4. Phosphatidylcholine-based liquid crystalline nanoparticles. Crystalline nanoparticles are drug crystals with PS ranging from dozens to a few hundreds of nanometres. The advantages of phosphatidylcholine-based liquid crystalline nanoparticles (PC-LCNPs) include non-toxicity, small size and considerable biocompatibility.133 PC-LCNPs were one of the candidates for controlled drug release. Tailoring the lipid membrane of the LCNPs by the PC molecules increased the nanoparticle elasticity and enhanced the drug penetration efficiency through the stratum corneum. PC-LCNPs are a new and attractive candidate for inner ear drug delivery applications with a significantly higher membrane surface area and applicable manufacturing process compared to elastic nano vesicles. Farrah et al. revealed the smart physicochemical properties of PC-LCNPs, as an attractive candidate as a caroverine carrier for non-invasive trans-tympanic delivery.105 Caroverine-loaded phosphatidylcholine-based nano elastic vesicles (EVs) were employed for comparison. Analysis of the solubilization efficiency (SE) showed a drug concentration of 0.25%, an optimum value. The decrease in SE at higher caroverine concentration resulted from the hydrophobic nature of caroverine. The entrapment efficiency increased with increasing concentration of T80 in the lipid/surfactant mixture from 10% to 20% and met decreases with a further increase. The vesicle mean diameter decreased with the increasing concentration of T80. The most desirable formulation with 150 mg of PC/T80 mixture containing 30% T80 was selected as the optimized formulation (OPT-EVs). Stable, non-lamellar PC-LCNPs were formed by the self-assembly of PC/glyceryl monooleate (GMO) in aqueous solution in the presence of stabilizers. Additionally, the PC-LCNPs formulation displayed good textural properties because of the unique bioadhesive nature of GMO. A test of the ex vivo transport of rabbit TM revealed a significant increase in the cumulative drug flux values of the PC-LCNPs compared to OPT-EVs.

Nevertheless, several demerits have limited the industrial application of vesicular systems, including low drug payload, physical instability of the vesicular bilayer and the sophisticated manufacturing techniques required for large batches.134

7.2. Chemical permeation enhancers

CPEs are defined as substances capable of promoting the penetration of drugs into the epithelium.135–139 The need for a drug to diffuse out of the carrier generally reduces the flux across the TM, and CPEs were introduced to help drugs cross the barriers.72 Invasive intratympanic applied CPEs were tested by Salt et al. through intracochlear injection or systemic delivery.140 Higher permeability of the RWM was significantly associated with a combination of dimethyl sulfoxide, N-methylpyrrolidone, saponin, and benzyl alcohol in observations by Salt et al.140 CPEs can reversibly increase the fluidity of the lipid bilayers in the interstitial space between impermeable corneocytes within the stratum adding an effect to provide a non-invasive way to deliver small molecular drugs across the intact TM.12,96–98 TDDS based on CPEs are generally cheaper than physical equipment. The synthesis of CPEs is uncomplicated compared to composite delivery systems of nanocarriers.

Khoo et al. formulated an in situ gelling system containing CPEs for non-invasive drug delivery into the middle ear (Fig. 5).12 In practice, Khoo et al. slowly added CPEs to a carrier–drug mixture including sodium dodecyl sulfate (SDS), limonene (LIM), and bupivacaine (BPV). The functions of CPEs (SDS, LIM, BPV) representing different chemical classes were tested.138 The outcomes demonstrated a marked improvement in the delivery of the antibiotic ciprofloxacin in the function of CPEs. The enhanced permeation can be maintained after incorporation into an in situ gelling matrix. A combination of all three CPEs was represented as 3CPE. Trans-tympanic flux experiments were performed to examine the effect of single and a combination of CPEs (no gel) on ciprofloxacin diffusion across the chinchilla TM. For this purpose, formulations containing ciprofloxacin and CPEs were administered to the EAC of live chinchillas. An alternative to increasing the concentration of a single CPE was to employ combinations of more than one CPE at lower concentration. The ciprofloxacin permeation produced by the three CPEs in combination was larger than that of the individual CPEs. The CPE-carrier formulation had minimal effect on auditory thresholds and tissue response in vivo. The major consideration with the application of CPEs is the balance between efficacy and toxicity.75,141,142

Yang et al. have described a trans-tympanic drug delivery system utilizing a liquid compound, with three CPEs: SDS, LIM, and BPV. The CPEs provided trans-tympanic ability to ciprofloxacin and were tested in a chinchilla animal model.96 Yang and colleagues further applied CPEs to enhance the flux of anaesthetics across the TM for the treatment of AOM.99 Methyl laurate (ML), another kind of CPE, was recently introduced by Liu et al. to form a single-CPE formulation with P407 and ciprofloxacin in the treatment of AOM in a chinchilla model.101

However, CPEs have the potential to damage the outer layer of the epithelium, leading to longer healing time. High permeation-enhancing potency has long been perceived to be associated with toxicity and potential for irritation.

7.3. Hydrogels

A hydrogel is a binary system containing polymers and liquid with cross-linking agents, forming 3D mesh structures. Absorbing and retaining a large amount of water inside, a hydrogel mimics the natural extracellular matrix with tuneable properties and versatile fabrication methods. Their great biocompatibility and biodegradability have made hydrogel materials promising candidates for a wide range of biomedical and engineering applications, including tissue engineering, regenerative medicine, and controlled drug delivery. Several injectable hydrogels were designed for middle and inner ear treatment.143,144 However, the application of various hydrogel systems for non-invasive trans-tympanic drug delivery requires more exploration.

A hydrogel can support spatial and temporal control over the release of various therapeutic agents. In the field of controlled
drug release, hydrogels serve as platforms for numerous physiochemical interactions between human tissue and encapsulated drugs, attributed to their tuneable physical properties, controllable degradability, and capability of protecting labile drugs from degradation.\textsuperscript{145} Trans-tympanic drug delivery should consider the spatial structure of the EAC and tissue permeability of the TM, indicating modified hydrogel delivery systems as alternative non-invasive therapeutic methods for middle and inner ear diseases.

### 7.3.1. Poloxamer 407

Poloxamer 407 (P407), namely Pluronic F-127, is an injectable synthetic hydrogel with a reversible mechanism for gelation. P407 is an ABA triblock polymer composed of a hydrophobic central block of polypropylene glycol (PPG) and two hydrophilic polyethylene glycol (PEG) ends.\textsuperscript{146–149} Considerable biocompatibility and biodegradability have been detected in P407. Based on its great solubilizing capacity, low toxicity, low cost, controllable drug-release characteristics, and compatibility with numerous biomolecules and chemical excipients, P407 has been widely used as an \textit{in situ} gel formulation for local drug delivery.

Convenience of application and quick gelling are needed in contacting TM for delivery. P407 is a thermosensitive polymer engaged in a sol–gel transition in response to temperature changes. P407 remains in a liquid state, facilitating ease of administration at lower temperature (below 25°C), and it experiences rapid gelation, transforming into a semi-solid gel form at body temperature. The temperature-triggered gelation of P407 is particularly applicable for trans-tympanic delivery, as it shows the characteristics of a favourable solution for topical use and a stable gel in contact with the TM. Yang \textit{et al.} have selected reverse thermal gelling hydrogels for trans-tympanic delivery (Fig. 8).\textsuperscript{96} Polymeric hydrogels have recently been developed to reversibly enhance drug delivery through the TM using CPEs.\textsuperscript{96} However, the interaction between the CPEs and P407 has an impact on the mechanical properties of P407 with a reduction in hearing. A P407–polybutylphosphoester (P407–PBP) with more hydrophobic domains was designed. Combining multiple medications in a hydrogel solution involves complex procedures which must be considered in manufacturing. Zhang \textit{et al.} designed PEGs in solution to investigate macromolecular permeation across the TM in a chinchilla \textit{ex vivo} model (Fig. 5).\textsuperscript{100} The delivery of macromolecules based on CPEs was tested. Three kinds of CPE were separately added: 1% SDS, 2% LIM, and 0.5% BPV. The permeation of the PEGs increased twenty-fold after the introduction of the 3 CPEs, suggesting the ability of the 3 CPEs to enhance permeability. The flux across the TM decreased with the growing molecular weight of the PEGs. Hydrogel systems based on P407 can promote the trans-tympanic flux of ciprofloxacin.\textsuperscript{96,98,99} The gel at the highest tested concentration contributed to 35% of the impedance to the flux across the TM. Gelation at body temperature leads to the reduction in flux by P407, slowing PEG diffusion within the gel.\textsuperscript{150}

Modification of P407 with other polymers may improve the sol–gel transition property of thermosensitive hydrogels in application as a trans-tympanic drug delivery medium. Budhori \textit{et al.} designed a series of thermosensitive norfloxacin reverse gelatinating gels composed of P407, Carbopol-940, and

![Fig. 8](https://example.com)
hydroxypropyl methyl cellulose (HPMC). Taking advantage of the individual properties of P407, Carbopol-940, and HPMC, a norfloxacin gel was developed to create a synergistic effect. P407 provides great thermosensitive characteristics, with the thickening and stabilizing properties of Carbopol-940 allowing controlled drug release and long-lasting gel in the EAC. HPMC was employed to enhance the flow behaviour and adhesive strength, contributing comfort and drug absorption to the applied gel system. P407 had a positive effect on entrapment efficacy. Formulation 10 exhibited the most favourable properties and achieved 95.6% drug release at 360 min in an ex vivo permeation study of a porcine oral mucosa model. A gel composed of P407, Carbopol-940, and HPMC has potential for the non-invasive controlled delivery of norfloxacin in the treatment of ear infections. The inclusion of P407 is essential for the convenience of administration and great adhesion to the intact TM.

However, there are significant limitations to thermosensitive P407, including insufficient gel strength, weak mucoadhesion, and rapid dissolution. The combination of P407 with other hydrophilic polymers or nanoparticles may modify the structure of P407 and overcome the drawbacks of P407 as a non-invasive topical drug delivery system.

Besides P407, there were other polymeric nanocarriers assessed in drug delivery to the middle and inner ear through invasive operation. PLGA, one component of P407, was used to form nanocarriers in gelfoam to be placed at the RWM for inner ear delivery by Tamura et al. PLGA nanocarriers can effectively permeate the RWM. The limitations of PLGA nanocarriers included: poor drug loading, high burst release, phagocytic uptake, and short half-life. Polyethylene glycol–polycaprolactone (PEG–PCL) diblock polymers encapsulating hydrophobic drugs were analysed by multiple invasive delivery methods for inner ear administration by Zhang et al. Trans-tympanic injection seemed to be an effective method of intracochlear drug delivery of PEG–PCL nanoparticles. A short half-life restrained the application of PEG–PCL nanoparticles as an intratympanic delivery medium. Amino acid and protein-based nanoparticles were biocompatible and increased drug retention time during delivery. Poly-amino acid-based nanoparticles such as poly(2-hydroxyethyl aspartamide) (PHEA) were used for inner ear delivery by Kim et al. through intracochlear injection. However, the production of PHEA-based materials was hampered by their unsatisfactory synthesis and low reactivity in terms of post-modification. In conclusion, surface medication and PS can significantly affect retention time, degradation rate, and permeability of the biological membrane to polymeric nanocarriers. Delivery methodology and functional interactions between nanoparticles and cells can impact the efficiency of administration. Several attempts were made to develop middle and inner ear invasive drug delivery systems by recruiting polymeric nanocarriers. The delivery efficiency of polymeric nanocarriers as non-invasive trans-tympanic drug delivery agents requires full elucidation.

7.3.2. Carbopol-940. As a crosslinked polyacrylic acid polymer, Carbopol-940 (known as a carbomer) is commonly used as a gelling agent in gel formulations. Carbopol-940 is a high-molecular-weight synthetic polymer with excellent thickening and gel-forming properties. Low toxicity and irritation potential were demonstrated in Carbopol-940. In systems with a prominent viscosity response, Carbopol-940 can be applied to thicken surfactant systems. Carbopol-940 played an important role in providing the desired consistency, viscosity, and stability in the norfloxacin gel formulation by Budhori et al. The function of Carbopol-940 was to enhance the mechanical strength of the gel and improve drug retention. Controlled drug release was achieved over an extended period by reduction of resistance during spreading. Additionally, the great stability of Carbopol-940 helped maintain the integrity of the thermosensitive gel product during storage and transportation. Carbopol-940 had a positive effect on the viscosity of the trans-tympanic reverse gelation gel. Nevertheless, an increasing concentration of Carbopol-940 caused a decrease in entrapment efficacy and percentage of drug release in the results of Budhori et al. Increasing the amount of Carbopol-940 limited water penetration in hydrogel systems, leading to a reduction in drug release.

7.3.3. Hydroxypropyl methyl cellulose. An HPMC is a polymeric compound containing repeating units of hydroxypropyl methylcellulose. The properties of an HPMC can vary significantly according to the molecular weight, the percentage of hydroxyl groups, the percentage of hydroxypropyl groups, and viscosity measurements. Based on its considerable thickening, gelling, low toxicity, and swelling properties, HPMC is commonly employed in the controlled-release formulation of both hydrophilic and hydrophobic drugs. HPMC was added to the trans-tympanic reverse gelation gel by Budhori et al. due to its ability to enhance rheological properties, providing pseudoplastic flow behavior. Smooth administration, convenient spreading, and excellent bioadhesion were reached in the application of HPMC, supporting prolonged contact, improved drug absorption and advanced therapeutic efficacy. Moreover, the moisture-retaining property of HPMC retained the appropriate hydration of the trans-tympanic gel, preventing gel shrinkage or cracking.

However, an increasing concentration of HPMC can reduce the amount of drug release as well as viscosity. The solubility of HPMC is impacted by numerous factors (such as molecular weight, degree of substitution, and temperature). The manufacturing process for HPMC is complex and requires precise control over several factors. The high viscosity grade of HPMC was compromised to produce a slower dissolution rate. The viscosity of HPMC hydrogel is influenced by various factors (such as temperature, pH, concentration, and shear rate), limiting its application in pharmaceutical manufacturing.

7.4. Other biomaterials with potential in non-invasive trans-tympanic delivery

Besides traditional nanocarrier delivery systems, several exploratory methodologies have been designed to complete non-invasive trans-tympanic drug delivery. Specific peptides were detected with high permeability through the TM. Active
delivery using a microshotgun was developed as an alternative for controllable release. Histamine, one mediator of allergy, has the potential to improve the permeability of the TM in particular delivery approaches.

7.4.1. Peptides. Epithelial barriers have mechanisms for transmucosal active transport across cells. Kurabi et al. investigated phage display to search for peptides able to cross the TM. \(^{17,106,107}\) Peptides with/without phages were transported across the TM with dependence on conditions of oxygen and temperature. \(^{70}\) The discovery of a trans-tympanic transport mechanism provides a potential approach for large-molecular weight drug delivery, gene therapy vectors and other macromolecules to treat middle ear diseases. The peptides and rates were divided into transport categories (high, medium high, and low) in a motif analysis by Kurabi et al. \(^{17}\) Secondary structural analysis indicated that the highest levels of trans-tympanic transport tended to occur between the +180°ψ and −180°ψ degree quadrants. More motif amino was found in the sequence of the highest transport rate category in tertiary structural analysis. The motifs would presumably be very available for binding to sites on the TM in the high transport peptide structure. Structural analysis of trans-tympanic peptides provided important information about the characteristics of transport rate, suggesting the relationship between a β-chain structure and high transport rate. The results predict interaction with protein substrates mediating active trans-tympanic transport. Furthermore, more motif amino acids were present in the sequence of the peptides in the highest transport rate category in a tertiary structural analysis. Para-cellular transport was regarded as a likely mechanism of large cargo transport in inhibitor studies. Transcytosis may exist in the TM.

Kurabi et al. further extended two of the candidate peptides with 6 additional amino acids at random. A second generation of peptides, the 18-mers libraries on TMs of rats for transport efficiency using phage display, were identified. \(^{18}\) Sequence analysis revealed anchor residues and structural features associated with enhanced transport. The results indicated that increasing the length of the 12-mer trans-tympanic peptides by 6 amino acids has the potential to significantly increase the transport rate. \(^{108}\) However, the mechanism of action is obscure. The binding partners on the TM involved in trans-tympanic transport were unknown. The analysis of trans-tympanic transport supports transcytosis as the mechanism of active transport of a cargo across the TM. The observation of Kurabi et al. provides a potential targeting mechanism for the delivery of therapies into the middle ear, including drugs, gene therapy vectors and bactericidal phages. However, the long-term safety and efficacy of trans-tympanic peptide transport should be locally and systemically tested. An immune response may be triggered by foreign peptides. An operating practice of safe long-term delivery was required with longitudinal investigation and optimization. There have been several recent studies employing peptides in the treatment of inner ear diseases, although the reported delivery methods were mostly invasive. \(^{156,157}\)

7.4.2. Microshotgun. The motion needed to cross the TM is one of the major challenges of trans-tympanic drug delivery. \(^{158,159}\) Current methods providing power consist of three types (biofuels, chemical fuels, and physical fuels). The limitations of current power generation include a short lifespan and the potential deposition of heavy metal silver and platinum in the body. Fabricating easy-to-prepare microtubes capable of providing sufficient power for nanoparticles is pivotal. Magnetophoresis has been applied to enhance drug permeation across biological barriers by applying an external magnetic field, such as in trans-tympanic delivery. \(^{160}\) A flexible micro-device of a “slim waisted” shape named a microshotgun was designed by Liang and coworkers as a drug delivery device with efficient trans-tympanic delivery (Fig. 9). \(^{13}\) The microshotgun consisted of nanoparticles, microtubes and a dry chemical propellant. The microtubes were produced by a layer-by-layer self-assembly method, taking advantage of positive or negative charges. The dry chemical propellants (organic acids or sodium bicarbonate) produced motion for the rapid release of bubbles by effervescent tablets, promoting the solid dispersion (SD) load into the microtubes. The wall of the microtube was comprised of Fe₃O₄-nanoparticles to achieve direction control in the magnetic field. The SD in the microshotgun can push the nanoparticles through the epithelial layer followed by acceleration by the magnetic field through the innermost layer of the TM. The trans-tympanic efficiency of the nanoparticles was improved by a two-stage design with dynamic data. However, actual drug delivery was not reported. The limitations of a microshotgun include a short lifespan and potential cytotoxicity. The complicated manufacturing process in a laboratory setting currently restricts production at scale for microshotguns. The trans-tympanic drug delivery efficiency of a microshotgun requires further observation.

An injectable and biodegradable thermosensitive hydrogel based on similar biomaterials was developed by Dai et al. for local protein delivery to the inner ear. \(^{161}\) However, the operational method was invasive with unclear drug distribution and elimination in the inner ear. Cai and coworkers proposed an effective PLGA nano-based strategy for enhanced drug delivery to inner ear tissue combining hydrophilic molecule-modified nanoparticles. \(^{162}\) The administration measure was injection, and the PLGA-based nanocarriers required a complex manufacturing process.

7.4.3. Histamine. Histamine is an organic nitrogenous compound involved in the local immune response communication and regulation of physiological functions. Histamine is mainly known for causing allergic inflammation disrupting the apical junctions of the epithelial barrier, leading to increased skin vascular and epithelial paracellular permeability. \(^{163}\) Histological structures of the skin and TM are similar. Inflammation triggered by histamine can increase the flux across the skin and shows potential applications in non-invasive trans-tympanic drug delivery. Zhang et al. developed a hydrogel formulation composed of P407, ciprofloxacin, CPEs, and proinflammatory histamine for trans-tympanic drug delivery in the treatment of a chinchilla otitis media model. \(^{109}\)
of histamine caused a 4-fold increase in the peak middle ear fluid ciprofloxacin level. An increase in middle ear fluid ciprofloxacin level was not detected in co-applied histamine with the gel. The results of Zhang et al. demonstrated that pre-treatment with histamine favoured the eradication of otitis media with ciprofloxacin. However, the pre-application methods limit the effectiveness of histamine in the treatment of AOM. Patients may not be able to expect the occurrence of disease two days in
8. Future directions

Non-invasive trans-tympanic delivery can operate without an uncomfortable experience or systemic side effects (Table 2). Various explorations have been made for potential biomaterials for non-invasive trans-tympanic drug delivery systems. However, several limitations require consideration in the future development of non-invasive trans-tympanic drug delivery systems (Fig. 3).

Firstly, the formulation of non-invasive trans-tympanic drug delivery systems should be able to pass through the irregular EAC and reach the outmost surface of the TM. To avoid additional requirements (e.g. specialized delivery equipment, training of operators, and overhead costs), delivery systems of

Table 2 Summary of essential components of non-invasive trans-tympanic delivery systems

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<th>Types of materials</th>
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<th>Applications</th>
<th>Delivery methods</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>Hydrogels</td>
<td>Poloxamer 407</td>
<td>Single hydrogel-forming polymer or one of the components in composite hydrogel</td>
<td>Ex vivo: deposited onto the TMs of harvested chinchilla auditory bullae①</td>
<td>Thermosensitive polymer with sol–gel transition under body temperature, rapid gelation</td>
<td>Insufficient gel strength, weak mucoadhesion, rapid dissolution</td>
</tr>
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<td></td>
<td>Carbopol 940</td>
<td>One of the polymers engaged in composite hydrogel</td>
<td>Ex vivo: hydrogel placed in the donor compartment to cross the membrane of porcine oral mucosa</td>
<td>Excellent thickening and gel-forming properties</td>
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</tr>
<tr>
<td>Hydroxypropyl methyl cellulose</td>
<td>Combined with polymers in composite hydrogel</td>
<td>Same as Carbopol 940</td>
<td></td>
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<tr>
<td>Nanocarriers</td>
<td>Nano-spanlastic vesicles</td>
<td>Thin-film-hydration technique used to prepare vesicles encapsulating ciprofloxacin</td>
<td>Ex vivo: formulation of vesicles placed in the EAC to cross the TM of sacrificed albino rabbits①</td>
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<td>Common interfacial agent material improving the shear strength and tensile strength</td>
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<td></td>
<td>Liposomes</td>
<td>Pneumococcal endolysin encapsulated in liposomes in PBS②</td>
<td>Ex vivo: donor chamber filter with sample for delivery across sheep and human TMs to receptor chamber</td>
<td>Great biocompatibility, incorporation of both hydrophilic and hydrophobic drugs</td>
<td>Short half-lives and low solubility</td>
</tr>
<tr>
<td></td>
<td>Nano-transfemoral vesicles</td>
<td>Ciprofloxacin-loaded transferosomes in PBS</td>
<td>In vivo: tested formulations applied otoprotically into the EAC of albino rabbits</td>
<td>Advanced flexibility and less rigid structure</td>
<td>Difficulty of loading hydrophobic drugs</td>
</tr>
<tr>
<td></td>
<td>Phosphatidylcholined-based liquid crystalline nanoparticles</td>
<td>Caroverine-loaded PC-LCNPs in diluted acetic acid solution③</td>
<td>Ex vivo: formulation from the EAC to cross the TM of albino rabbits to the receptor</td>
<td>Non-toxicity, considerable biocompatibility, ability of controlled release</td>
<td>Low drug pay load, physical instability</td>
</tr>
<tr>
<td>Chemical permeation enhancers</td>
<td>Sodium dodecyl sulfate, limonene, bupivacaine</td>
<td>CPE and ciprofloxacin-loaded poloxamer 407 hydrogels④</td>
<td>In vivo: CPE-P407 formulation administered to the EAC of live chinchillas⑤</td>
<td>Increasing permeability across the epithelium</td>
<td>Potential to destroy the outer layer of the epithelium</td>
</tr>
<tr>
<td>Peptides</td>
<td>Specific peptides with basic amino acid at position 6, and strongly hydrophobic residues at the C-terminus</td>
<td>NA⑥</td>
<td>NA</td>
<td>Potential approach for large-molecular weight drug delivery</td>
<td>The mechanism of transmission is obscure as well as their long-term safety</td>
</tr>
<tr>
<td>Microshotgun</td>
<td>Fabricated by a micro-porous template-assisted layer-by-layer assembly method</td>
<td>NA</td>
<td>NA</td>
<td>Acceptable biocompatibility, target penetration in magnetic field, high efficiency, easy-to-prepare</td>
<td>The actual drug delivery efficiency not reported, short lifespan, potential cytotoxicity</td>
</tr>
<tr>
<td>Organic compounds</td>
<td>Histamine</td>
<td>Histamine solution</td>
<td>Ex vivo: pre-application of histamine solution onto the chinchilla TM before the administration of ciprofloxacin-loaded P407</td>
<td>Inflammation increasing flux across the epithelium</td>
<td>Pre-application method not applicable in clinical circumstances</td>
</tr>
</tbody>
</table>

① Abbreviations: TM, tympanic membrane, EAC, external auditory canal, PBS, phosphate buffered saline, PC-LCNP, phosphatidylcholine-based liquid crystalline nanoparticles, CPE, chemical permeation enhancers, P407, poloxamer 407, NA, not applicable.
liquid forms were preferred over other forms of formulation (such as solid) to accommodate the cavity shape of the irregular EAC and stick to the TM. Both administration practice and the practice of large-scale manufacturing should be well designed to produce a balance between practical viability and standard production.

Moreover, gelation and the adhesion of applied delivery systems to the TM can support controllable drug release for long-term delivery. Conventional ototopical drops can easily flow away immediately after administration and demand multiple deliveries daily. Frequent operation is difficult to ensure, especially in paediatric patients, those mainly vulnerable to AOM. Gelation of liquid delivery systems once attached to the TM can provide a long-term delivery approach. The permeative efficiency of a drug is associated with the contact area between the formulation and the TM. The application of a thermosensitive hydrogel may help enlarge the acceptable area when the delivery system completes the sol–gel transition in contact with the TM.

Secondly, the non-invasive approach requires further research because of the unsatisfactory concentration of therapeutics permeating across the intact TM and RWM. The development of TDDS can be referred to with modification. The excellent transdermal permeability of various nanocarriers (e.g. polymeric nanocarriers and lipid nanoparticles) has been evaluated. Active delivery and passive delivery can both be applied to increasing the permeability of a delivered drug through the TM and the RWM. The enhancement of transdermal delivery by equipment (active delivery) accelerates the therapeutic efficacy of delivered drugs. A microshotgun used a magnetic field force to guide the fast permeation of nanoparticles through the intact TM. There was a recent development of chemical enhancers (passive delivery) increasing the spread of drugs across the skin or increasing the solubility of drugs in the skin. Polymeric nanocarriers and CPEs were evaluated in a non-invasive trans-tympanic drug delivery system for middle and inner ear diseases.

Drug penetration across the TM and RWM is influenced by many factors, including temperature, age, state of the TM or RWM and the physical characteristics of the penetrant. The localized conditions of the middle and inner ear vary according to different circumstances and individuals, including temperature and middle ear pressure. Non-invasive trans-tympanic delivery requires adaption to various physiological conditions (such as a fluid-filled middle ear in OME), which is especially critical for methods accommodating the changing local physiological environments. Inner ear diseases can be more severe and more intractable than middle ear conditions. Further technical refinement is needed for successful drug diffusion into the middle and inner ear.

Thirdly, maximizing drug concentration and minimizing adverse effects are required for drug delivery systems in the middle and inner ear space. The purpose of drug-loaded nanocarriers is to achieve controllable long-term delivery in the middle and inner ear. Conventional liquid formulations are quickly eliminated from the middle and inner ear. Drug encapsulation into nanocarriers has been developed to overcome the unstable metabolism inside the middle and inner ear. Biomedical nanocarriers are well known to sustain drug release and protect the effective substances from degradation. Drug delivery systems designed for non-invasive trans-tympanic administration should meet the following specifications: sufficient drug at the target site, the avoidance of rapid clearance, and acceptable toxicity. The biocompatibility and biodegradability of the delivery system are important. The potential of a foreign object inflammatory response should be avoided. A high concentration of a drug may favour trans-tympanic gradient diffusion while increasing the adverse tissue effects at the TM and EAC. There is a need to test the long-term tissue reaction of delivery systems, especially if non-biodegradable materials are involved.

The pharmacokinetics of drug delivery are hard to monitor due to the difficulty of microenvironmental inspection of the middle and inner ear. Additionally, the delivery efficacy of innovative biomaterials was doubtful. Validation of the therapeutic efficacy and reduction of potential toxicity of non-invasive systems are important in pre-clinical assessment. In the field of non-invasive trans-tympanic drug delivery systems, there is a real demand for validated evaluation methods measuring pharmacokinetics and biosecurity.

9. Conclusions

A better understanding of the anatomy and physiology of the middle and inner ear will enable the design of tunable non-invasive drug delivery systems of middle and inner ear diseases. Liquid formulations can easily pass the EAC and contact the intact TM. Thermosensitive hydrogels increase the effective release time when they have completed the sol–gel phase change at the outermost surface of the TM. Both active and passive delivery mechanisms were employed in non-invasive trans-tympanic administration, referring to the current development of TDDS. Controllable release and pharmacokinetics were considered in polymeric nanocarriers and lipid nanoparticles for middle and inner ear treatments. Further study may focus on modifications improving diffusion efficiency, adaptability, and convenience of practice. Marketed middle and inner ear delivery platforms are promising for handling middle and inner ear ailments.

Ethics approval and consent to participate

There are no human and animal subjects in this review and informed consent is not applicable.

Author contributions

Yang Xu: writing, original draft preparation; Zhongwu Bei: original draft preparation; Ke Qiu: review and editing; Mei Li: review and editing; Jianjun Ren: review and editing; Bingyang
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