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Evaluation of intratympanic formulations for inner ear delivery: methodology and sustained release formulation testing

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Abstract

A convenient and efficient *in vitro* diffusion cell method to evaluate formulations for inner ear delivery via the intratympanic route is currently not available. The existing *in vitro* diffusion cell systems commonly used to evaluate drug formulations do not resemble the physical dimensions of the middle ear and round window membrane. The objectives of this study were to examine a modified *in vitro* diffusion cell system of a small diffusion area for studying sustained release formulations in inner ear drug delivery and to identify a formulation for sustained drug delivery to the inner ear. Four formulations and a control were examined in this study using cidofovir as the model drug. Drug release from the formulations in the modified diffusion cell system was slower than that in the conventional diffusion cell system due to the decrease in the diffusion surface area of the modified diffusion cell system. The modified diffusion cell system was able to show different drug release behaviors among the formulations and allowed formulation evaluation better than the conventional diffusion cell system. Among the formulations investigated, poly(lactic-co-glycolic acid)–poly(ethylene glycol)–poly(lactic-co-glycolic acid) triblock copolymer systems provided the longest sustained drug delivery, probably due to their rigid gel structures and/or polymer-to-cidofovir interactions.

Keywords

Drug release; inner ear delivery; intratympanic formulation; methodology; temperature; responsive gel

Introduction

Drug delivery to the ear for the treatment of inner ear disorders has been an area of considerable interest to both researchers and clinicians in the past decades^{1,2}. Hearing loss such as sensorineural hearing loss (SNHL) is a result of damages to the inner ear cells or

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Declaration of interest

There is no other declaration of conflict of interest.

auditory nerves caused by trauma noise, aging, medication, chemical and radiological therapy, and heredity. Other common ear disorders include ear infections, tinnitus, Meniere's disease and vertigo. Among the inner ear disorders in young populations, SNHL caused by congenital infections of cytomegalovirus (CMV) are common in newborns, and antivirals used in the treatment of CMV such as ganciclovir and cidofovir can be effective in preventing SNHL^{3,4}. Cidofovir is the second drug in line for CMV infection particularly with patients who have developed ganciclovir resistance. Thus, the development of an effective drug delivery system for inner ear using cidofovir can lead to better management of SNHL.

Systemic drug delivery of medications to the inner ear for either controlling the symptoms of hearing disorders or reducing the damage to the inner ear cells is not effective due to the blood-cochlear barrier and adverse effects associated with the high drug concentration in the systemic circulation^{2,5}. Local drug delivery such as intratympanic injection is more effective than systemic administration, and has been investigated in the treatments of a variety of inner ear diseases. In intratympanic delivery, drugs in formulations are delivered through the tympanic membrane to the middle ear (Figure 1a), from which the drugs permeate through the round window membrane (RWM) into the inner ear by passive diffusion. For example, a number of clinical studies have been published on intratympanic injection of corticosteroids in the treatments of Meniere's diseases⁶⁻⁸ and SNHL⁹⁻¹¹. Despite the common usage of intratympanic injection, its clinical utility is limited due to the variable clinical outcomes in the treatment of inner ear diseases via this route. This is partly due to the variable contact duration of the drug solution in the middle ear with the RWM^{12,13}. Several formulation approaches to increase the residence time of drugs in the middle ear have been proposed to improve inner ear delivery, and the use of sustained release formulations has shown some promise¹⁴⁻¹⁸. Among these formulations, temperature responsive polymer systems could be an effective sustained release strategy for intratympanic drug delivery to the inner ear.

In formulation development, a new formulation and its drug release profile can be tested in diffusion cell settings *in vitro*¹⁹⁻²¹, and pharmacokinetics and/or its duration of action can be examined in animals *in vivo* afterwards. The main purposes of the *in vitro* diffusion cell release studies are to characterize the physical properties of the drug formulations and to provide batch quality control in manufacturing. The ability of the *in vitro* testing method to predict *in vivo* results through *in vitro/in vivo* correlation is also preferred²². Ideally, the development of an *in vitro* testing method should be based on the knowledge of the *in vivo* conditions in which drug release occurs in the body. The *in vitro* drug release testing method should mimic the physiological conditions *in vivo*. At present, in the case of testing intratympanic drug delivery systems, no standard testing method has been established for the evaluation of inner ear delivery. The existing *in vitro* diffusion cell systems such as Franz diffusion cells commonly used to evaluate topical and transdermal formulations do not resemble the physical dimensions of the middle ear and RWM for formulation development. A convenient and efficient *in vitro* diffusion cell method to evaluate intratympanic formulations for inner ear delivery is not available. Further research and development in this area are needed.

The objectives of this study were (a) to evaluate a new *in vitro* diffusion cell setup for studying intratympanic drug delivery systems such as in formulation screening and (b) to identify the parameters required for effective sustained drug delivery under the physiological conditions such as the small enclosure of the middle ear and surface area of the RWM encountered in inner ear delivery. The drug release profiles of four temperature responsive drug delivery systems were compared in the conventional diffusion cell setup and the present modified diffusion cell setup *in vitro*. Cidofovir was chosen as the model drug in this study because of its antiviral property and efficacy in the treatment of congenital infections due to CMV.

Methods

Materials

Cidofovir injection solution (Vistide, 75 mg/mL) was from Gilead Sciences (Foster City, CA). The drug powder was obtained by the lyophilization of the cidofovir injection solution and stored at 4 °C before use. Pluronic® F 127 Prill was purchased from BASF Chemical Company (Mt. Olive, NJ). Poly(lactic-co-glycolic acid)–poly(ethylene glycol)–poly(lactic-co-glycolic acid) triblock copolymer (PLGA–PEG–PLGA; AK24, of PEG 1000 and 3:1 lactide:glycolide, $M_N = 3552$ and $M_W = 4286$) was purchased from Akina, Inc. (West Lafayette, IN). Poly(ethylene glycol) of molecular weight 1450 (PEG 1450), DL-lactide and glycolide were purchased from Polysciences Inc. (Warrington, PA). Stannous 2-ethyl hexanoate was purchased from Sigma-Aldrich (St. Louis, MO). Tetrabutylammonium phosphate monobasic and sodium phosphate dibasic heptahydrate were purchased from Acros Organics (Fair Lawn, NJ). High-performance liquid chromatography (HPLC) grade acetonitrile was purchased from Pharmaco-AAPER (Shelbyville, KY). Phosphate-buffered saline (PBS, pH 7.4, consisting of 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride) was prepared by dissolving PBS tablets (Sigma-Aldrich, St. Louis, MO) in distilled, deionized water.

Synthesis of PLGA–PEG–PLGA copolymer

In addition to the commercial PLGA–PEG–PLGA copolymer, a second PLGA–PEG–PLGA copolymer (PEG 1450 and 8:1 lactide:glycolide) was synthesized and tested in this study. This PLGA–PEG–PLGA copolymer was synthesized using a method similar to that described previously²³ with modifications. Briefly, PEG 1450 (1.5 g) in 100 mL round bottom flask was dried under vacuum with stirring at 120 °C maintained with sand-bath for 3 h. Then, 3.17 g DL-lactide and 0.32 g glycolide were mixed with PEG 1450 in the flask and the mixture was heated at 120 °C under vacuum for 30 min. After all the materials in the flask were melted, stannous 2-ethyl hexanoate was added to the flask, and the mixture was heated to 155 °C for 8 h. At the end of the reaction, the purification of the copolymer was performed by alternate cooling and heating of the mixtures in the flask. In the purification, the copolymer obtained in the flask was dissolved in cold water (5–8 °C). After complete dissolution, the copolymer solution was heated to 80 °C to precipitate the copolymer. The precipitated copolymer was collected by decanting the supernatant, and the copolymer was then dissolved in cold water again. This procedure was repeated three times. The copolymer obtained after purification was freeze-dried to yield a viscous semisolid polymer.

Preparation of formulations

Table 1 lists the four formulations and control investigated in this study. The transition temperatures of these formulations were determined by physical observation in a temperature-controlled oven or cold water-bath. The concentration of cidofovir in all the formulations and control was 5 mg/g. PBS or water was used as the vehicle in the preparation. First, cidofovir solution was prepared by dissolving freeze-dried cidofovir powder in PBS and was used as a solution control. PLGA-PEG-PLGA formulations (1 g) of 20% (w/w) commercial and 10% (w/w) “in-house” synthesized PLGA-PEG-PLGA copolymers were prepared by mixing 200 mg of the commercial or 100 mg synthesized PLGA-PEG-PLGA, 0.733 or 0.833 mL PBS, respectively, and 0.067 mL of 75 mg/mL cidofovir injection solution in a rotary mixer at room temperature (20–24 °C) or in a cold room (~4 °C), respectively, for 48 h and then stored at 4 °C in a refrigerator until use. Pluronic formulations (20% and 30% poloxamers, w/w, respectively) were prepared by adding the required amounts of Pluronic granules to 5 mg/g cidofovir in water at 4 °C until all the granules were completely dissolved and a clear solution was obtained. The formulations were then stored at 4 °C until use.

In vitro release studies

In vitro release experiments were performed using two different diffusion cell setups: conventional Franz diffusion cells and modified diffusion cells (Figure 1b and c, respectively). In the conventional diffusion cell setup, a cellulose membrane (0.45 µm; Millipore, Billerica, MA) was used to separate the donor and receptor chambers. The conventional diffusion cell setup had an opening of 0.2 cm² (a diameter of approximately 5 mm) as an effective area for diffusion (or drug release from the formulation in this case). In the modified diffusion cell setup, an assembly of a silicone membrane (0.5 mm in thickness) with a small opening of approximately 0.3 mm² (a diameter of 0.6 ± 0.1 mm, mean ± SD) and a cellulose membrane (0.45 µm; Millipore) was used to separate the donor and receptor chambers. The small opening was located at the center of the silicone membrane that was mounted on top of the cellulose membrane with the silicone membrane facing the test formulations in the modified diffusion cells. At the start of the *in vitro* release experiments, 0.1 mL test formulation was applied to the donor chamber of the diffusion cells, and the receptor chamber was filled with 5 mL PBS with 0.2% sodium azide as a preservative. The donor chamber was then sealed with Parafilm. The experiments were performed under constant stirring in the receptor chamber at 37 ± 1 °C maintained by a circulating water-bath. At predetermined time intervals, 1.0 mL samples were withdrawn from the receptor chamber for cidofovir assay using HPLC, and an equal volume of fresh receptor solution was immediately added back to the chamber to maintain a constant volume in the receptor. The durations of the release experiments ranged from 48 to 96 h. At the end of experiments, the donor chamber was rinsed with PBS and the rinsing solution was collected to determine the amount of residual drug in the donor chamber. All release experiments were carried out in at least quadruplicate.

HPLC analysis of cidofovir

HPLC analyses were performed using a liquid chromatography system (Prominence, Shimadzu, Columbia, MD) equipped with CBM-20A system controller, LC-20AT solvent delivery module, SIL-20A autosampler and SPD-20A UV-Vis detector. The separation was performed on an Eclipse XDB-C8 column (150 × 4.6 mm, 5 μm; Agilent Technologies, Santa Clara, CA) for the assay of cidofovir. The mobile phase consisted of 3.5 mM sodium phosphate dibasic and 1.5 mM tetrabutylammonium phosphate monobasic in water and acetonitrile at a ratio of 90:10 (v/v). The mobile phase was delivered at a flow rate of 1.0 mL/min and the detection wavelength was 274 nm. Calibration curves were constructed using standards of cidofovir concentrations between 1.5 and 75 μg/mL and found to be linear in this range.

Computer model simulations

Finite-element simulations of drug diffusion were set up to study the effect of diffusion area of the conventional and modified diffusion cell systems upon drug release and to identify the parameters that may lead to the differences in the drug release profiles observed in these two diffusion cell systems. Computer simulations were performed using COMSOL Multiphysics® software version 4.2 (Comsol, Inc., Burlington, MA) based on Fick's second law:

$$\frac{\partial C}{\partial t} = D \nabla^2 C \quad (1)$$

where ∇ is the vector differential operator, C and D are the concentration and diffusion coefficient of the drug in the formulation, respectively, and t is the time. In the model simulations, the geometry of the diffusion cell donor chamber (Figure 1d) was represented by an axially symmetric two-dimensional rectangle with dimensions of 2.5 mm × 2.5 mm (to form a cylinder with 2.5 mm height × 5.0 mm diameter). One edge of the rectangle, 2.5 mm in length, represents the radius of the diffusion area of the Franz diffusion cell setup (diameter = 5.0 mm). Simulations were performed using a time-dependent diffusion model. The percent of drug remaining in the rectangle was calculated by the concentration of the drug (in mol/mm²) at time $t = 0$ compared to the concentration of the model drug at time t . In simulations representing the conventional Franz diffusion cell, the diffusion of the model drug occurred along the entire 2.5 mm edge of the rectangle. In simulations representing the modified Franz cell, the diffusion of the model drug out of the rectangle occurred on the edge of a small region of 0.20 mm × 0.60 mm, 0.25 mm × 0.60 mm or 0.30 mm × 0.60 mm extended from the rectangle along the axis of symmetry (Figure 1e), representing the radius and length of the small pore in the silicone membrane (pore diameter = 0.40, 0.50 or 0.60 mm, respectively, and length = 0.6 mm). Drug interactions with the formulations were modeled by modifying the diffusion coefficient of the drug in the formulations. Diffusion coefficients of 2.3×10^{-6} , 4.5×10^{-6} and 9.0×10^{-6} cm²/s were used, and the effect of drug diffusion rate in the formulations as it relates to drug release into the receptor chamber was examined.

Statistical analysis

All data were presented as their mean $\pm$ standard deviation (SD). One-way ANOVA Tukey tests were performed using GraphPad InStat (San Diego, CA) to compare the experimental data. Linest calculations (Microsoft Excel, Redmond, WA) were performed to determine the slopes and standard errors (SE) in the least squares regression analyses. Statistical differences were considered to be significant at a level of $p < 0.05$.

Results

Physical properties of the temperature responsive polymer systems

Table 1 lists the physical properties of the temperature responsive polymer systems tested for sustained drug delivery in this study. Cidofovir is readily soluble in water. The phase transition temperatures of the formulations ranged from 9 to 35 °C. The formulations were solutions below these transition temperatures and turned into gels above these temperatures.

In vitro release of cidofovir with the conventional diffusion cells

The cumulative percentage release of cidofovir from the temperature responsive formulations and reference control was monitored for up to 72 h in the conventional Franz diffusion cell system. Figure 2 shows the *in vitro* drug release profiles of the formulations. In the initial 6 h, significant amounts of the drug were released from all the systems studied. Particularly, for the Pluronic and PLGA–PEG–PLGA formulations, approximately 60–80% of the loaded cidofovir was released in the first 6 h and over 90% of the drug was released in 2 d. As expected, the solution system (control) exhibited the fastest drug release profile of all the systems studied.

The formulations in the donor chambers were also examined at the end of the release experiments. Whereas the Pluronic hydrogels (20% and 30%) sometimes dissolved completely and their residues in the donor chambers were in liquid state, the commercial and synthesized PLGA–PEG–PLGA hydrogels remained as semisolids in the donor chambers.

In vitro release of cidofovir with the modified diffusion cells

The cumulative percentage release profiles of cidofovir from the temperature responsive formulations and control in the modified diffusion cells with a small diffusion area (Figure 3) were different from those obtained with the conventional diffusion cells (Figure 2). This difference can be attributed to the difference in the diffusion area of the diffusion cells. In general, drug release in the modified diffusion cells was slower than that in the conventional diffusion cells. Cidofovir solution (control) showed the fastest rate of drug release in the modified diffusion cells (~60% in 2 h), which was slower than that observed in the conventional diffusion cell system (~94% in 2 h). For 20% and 30% Pluronic hydrogel systems in the modified diffusion cells, only 15–20% drug were released in the first 6 h, and the drug release was sustained for over 2 d. Among the systems studied, the slowest drug release was observed when cidofovir was incorporated in the PLGA–PEG–PLGA formulations. Although the release of cidofovir was almost complete with the Pluronic formulations at 96 h in the modified diffusion cells, only 46 to 70% drug was released from the PLGA–PEG–PLGA formulations in the same time period. For the appearance of the

formulations in the donor chambers at the end of the release experiments, similar results were observed in the modified diffusion cells as those in the conventional diffusion cells.

Finite-element computer simulations

The difference between the release profiles in the conventional and modified diffusion cell experiments can be explained by the different diffusion areas of the diffusion cell setups. Although drug release occurs through the whole diffusion area of the conventional diffusion cells, the small diffusion area of the modified diffusion cells is the rate-limiting diffusion region for drug release from the sustained release formulations into the receptor chamber. Figure 4 shows the model simulation results of drug release in the diffusion cell systems of various diffusion areas. When the size of the diffusion area decreased, drug release was prolonged. In addition to the surface area, as expected, the diffusion coefficient of the drug in the formulation also affected the rate of drug release and hence the sustained release effect: when the diffusion coefficient decreased, the rate of drug release decreased. The simulation results confirm that the experimental results obtained in the present drug release experiments follow the trends that one would expect from fundamental diffusion theory.

Another useful aspect of the finite-element simulation study was to analyze the release profiles of the simulation results with the simple first-order release model:

$$\ln \left(\frac{C_t}{C_0} \right) = -kt \quad (2)$$

where C_t is the concentration of the drug remaining in the donor chamber at time t , C_0 is the initial concentration of the drug and k is the apparent first-order release rate constant. Drug release into the receptor chamber would follow first-order release kinetics when drug diffusion across the filter membrane at the diffusion cell opening is the rate-limiting step. In these analyses, the concentration of the drug remaining in the donor chamber C_t was first calculated by the drug release data in Figure 4. The ratio C_t/C_0 was then analyzed in a semi-log plot using Equation (2). Figure 5 presents the representative semi-log plots used in these analyses. For the modified diffusion cells, the drug release profiles can generally be described as first-order release with reasonable coefficients of determination ($R^2 > 0.999$). For the conventional diffusion cells, due to the error in the assumption that diffusion across the diffusion cell opening is the rate-limiting step in the first-order release model, the fittings of the drug release profiles to the first-order kinetics are poorer (R^2 ranging from 0.992 to 0.999) than those of the modified diffusion cells.

Discussion

Conventional and modified diffusion cells

Although *in vitro* diffusion cell testing generally cannot be used to predict bioavailability *in vivo*, it is a useful technique for the characterization of drug delivery systems and for product quality control in manufacturing^{24,25}. In the present *in vitro* drug delivery testing, as predicted from diffusion theory in the model simulations, the diffusion coefficients of the drug in the formulations and the diffusion areas for drug release can significantly affect drug release from the formulations, and hence resulting in the different drug release profiles

observed among the different formulations and between the conventional and modified diffusion cells. In addition, the results in the finite-element computer model simulations show that drug release in the modified diffusion cell study can be assumed to be first order, probably due to diffusion across the small diffusion area of the modified diffusion cells being the transport rate-limiting step of drug release from the donor chamber. From a practical viewpoint, the smaller diffusion area of the modified diffusion cells than the conventional diffusion cells is better representation of the physiological dimensions for inner ear delivery. The RWM is a soft tissue barrier separating the inner ear from the middle ear and is regarded as the main route for drug passage from the middle ear cavity into the inner ear in the otic capsule. The RWM is small (approximately 1 mm² in size in guinea pigs and 2.5 mm² in adult humans) and is located within the round window niche. Because of the small diffusion area of the RWM passage, the conventional diffusion cell setup may not be adequate in the assessment of intratympanic formulations for inner ear delivery. This concern is illustrated by the different results obtained in the modified diffusion cell and the conventional diffusion cell settings in this study; drug release was prolonged from the sustained release formulations when the diffusion surface area of the diffusion cells decreased.

To further compare the drug release data, the drug release profiles were analyzed using Equation (2) under the first-order release assumption, and the results are presented in Table 2. In general, drug release in the modified diffusion cells can be described as first order (R^2 ranging from 0.954 to 0.999) better than the conventional diffusion cells (R^2 ranging from 0.840 to 0.998). The results in the table also show that the apparent first-order release rate constants in the modified diffusion cells are 3–30 times smaller than those in the conventional diffusion cells for the sustained release formulations. This can be attributed to the smaller diffusion area of the modified diffusion cells although a quantitative correlation between the changes in the apparent rate constants and diffusion cell opening areas is lacking. A possible explanation of this discrepancy is the error in the assumption of first-order release for the conventional diffusion cells. In addition, drug release in the diffusion cell experiments is expected to be more complicated than simple diffusion in the model because drug release in the experiments could be affected by (a) water movement from (or into) the receptor to (or from) the donor, leading to convective transport, (b) water evaporation from the formulation in the donor during the experiments, (c) alteration of the copolymer concentration in the donor over the duration of the experiments due to such water movement or evaporation, resulting in a change in physical properties of the gel in the donor such as dissolution of the gel and (d) possible interactions between the drug and copolymers and non-Fickian diffusion.

Another observation of the results in Figures 2 and 3 and Table 2 is the more distinctive drug release profiles of the formulations in the modified diffusion cell system relative to the conventional diffusion cell system. Particularly, both the commercial and synthesized PLGA–PEG–PLGA formulations show significantly lower apparent release rate constants compared to the Pluronic formulations in the modified diffusion cells ($p < 0.01$, ANOVA), whereas the apparent release rate constants of PLGA–PEG–PLGA and Pluronic formulations are not significantly different in the conventional diffusion cells ($p > 0.05$).

This suggests that diffusion cells with smaller diffusion area can provide better assessment in formulation evaluation under the present conditions. Conventional Franz diffusion cell systems may not be sensitive enough to be a product qualifying tool to evaluate formulations in inner ear drug delivery.

It should be noted that the drug release results in this study can also be investigated using a receding boundary release (or square root of time release) model that is commonly used to describe drug release from suspensions, semisolids and granular matrices. The analyses in the present study have mainly focused on the first-order release model because the fittings of the square root of time release model (i.e. drug release versus square root of time plots) are relatively poor for the experimental and computer simulation data (Figure 6) compared to the first-order release model (Figure 5b) with the modified diffusion cells. Changing the dimensions of the donor formulation in the computer simulations, e.g. increasing the donor volume by two-fold, did not affect the conclusion of these fittings (data not shown).

Temperature responsive drug delivery systems for inner ear delivery

Thermosensitive polymers have attracted interest in drug delivery applications due to their ease of administration and minimal invasiveness²⁶. Typically, biodegradable thermosensitive polymers are block copolymers composed of hydrophobic and hydrophilic polymer blocks²³. The sustained release systems employed in this study have transition temperatures around body temperature to provide temperature responsive transition after injection. Among these formulations, the PLGA-PEG-PLGA systems have the slowest drug release profiles (lower apparent first-order release rate constants, $p < 0.05$). This may be due to the rigid structures of the PLGA-PEG-PLGA polymers in gel phase that the solid gels maintained their physical forms over the entire duration of the drug release experiments; PLGA-PEG-PLGA gels did not dissolve and diffuse into the receptor chamber and had more rigid physical forms compared to those of Pluronic. Another possible explanation is specific interactions between cidofovir and PLGA-PEG-PLGA polymers that decreased the effective diffusion coefficients of cidofovir in the PLGA-PEG-PLGA formulations and hence decreased the release rates of cidofovir from the gels. This result is consistent with the observation of slower drug release from PLGA-PEG-PLGA than Pluronic in a previous study of paclitaxel²⁷. With the sustained release of cidofovir from the PLGA-PEG-PLGA formulations for three or more days in the modified Franz diffusion cells using Millipore filter membrane in the present study, it is expected that the duration of drug delivery across the RWM with these formulations would be longer than those in this study because the permeability of the RWM is likely to be lower than that of the Millipore filter; the RWM is a semipermeable membrane^{28–30}. The use of intratympanic formulations with the ability to provide sustained release of cidofovir such as PLGA-PEG-PLGA can be significant in the prevention and treatment of SNHL caused by CMV in the inner ear.

Inner ear delivery in practice

In inner ear drug delivery, it has been suggested that the effectiveness of inner ear drug delivery through the intratympanic route mainly relies on the duration of the drug in contact with the RWM³¹. In intratympanic injection of drug solution into the middle ear, a large portion of the administered drug is eliminated from the middle ear through the Eustachian

tube³². This makes intratympanic drug delivery highly variable. Efforts to control this variability and to improve inner ear delivery following intratympanic injections have led to the development of sustained release vehicles for inner ear drug delivery. Although sustained release delivery systems have been studied extensively in other routes of administrations, these systems have only been recently investigated for inner ear delivery². *In vitro* methods to study inner ear delivery systems are also limited with only a handful of reports in the literature of methods such as drug release in containers^{15,33}, excised temporal bones and organotypic explant cultures³⁴, cell culture on membrane inserts in multiple well plates³⁵, cell layer membrane model³⁶ and conventional Franz diffusion cells³⁷. A standard *in vitro* testing method using diffusion cells to evaluate drug formulations such as sustained delivery systems for the inner ear is not available. The results in this study suggest the need of reducing the diffusion area of vertical diffusion cells to mimic the physical dimensions of the RWM as the *in vitro* testing method for inner ear delivery.

This study also provided insights into the factors governing sustained drug delivery to the inner ear. Because the middle ear is enclosed in the temporal bone with relatively impermeable bone surrounding, drug clearance in the middle ear occurs predominately through the Eustachian tube with a small portion of the drug delivered into the inner ear via the RWM. According to the results in this study, if the drug formulation can stay in the middle ear in contact with the RWM for a prolonged period of time, sustained drug delivery can be accomplished even with a formulation that does not demonstrate substantial sustained release properties in the conventional diffusion cells due to the small diffusion surface for drug release across the RWM. This suggests that the ability of a sustained release formulation to remain in the middle ear is likely more important than drug diffusion coefficient in the formulation (or the resultant rate of drug release from the formulation), so a sustained release system with rigid physical form and adequate mechanical strength is preferred. In addition to enhancing the amount of drug delivered to the inner ear, sustained drug delivery can also lead to more uniform drug distribution in the cochlea. Computer simulations in a previous study have shown that the duration of drug application can significantly affect both drug concentration in the cochlea and drug concentration gradient along the length of the cochlea³⁸. Particularly, higher drug concentration and smaller drug concentration gradient in the cochlea were observed with prolonged 24-h drug delivery on the RWM versus those of 30 min (e.g. drug delivery by a sustained delivery system versus that following a single injection application of drug solution), but the effect of the duration of drug delivery becomes less significant from 24 h to 7 d. This illustrates the advantages of sustained drug delivery and the potential of the present PLGA-PEG-PLGA formulations, which can provide sustained drug release across a small diffusion surface for 3 d, for intratympanic inner ear delivery.

Conclusion

A modified *in vitro* diffusion cell system was employed in this study to evaluate sustained release formulations for inner ear drug delivery. The main modification of this *in vitro* diffusion cell system was its diffusion area that was a better representation to the physical dimensions of the RWM than the commercially available *in vitro* Franz diffusion cell systems. As anticipated from diffusion theory, it was shown that drug release from the

formulations in the modified diffusion cell system was slower than that in the conventional diffusion cells and sustained drug delivery can be easier to accomplish with the smaller diffusion area. The modified diffusion cell system was also more sensitive in differentiating the drug release kinetics of sustained release formulations than the conventional diffusion cells. Among the formulations investigated in this study, the triblock copolymer PLGA–PEG–PLGA systems provided the longest sustained drug delivery.

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References

1. Borenstein JT. Intracochlear drug delivery systems. *Expert Opin Drug Deliv.* 2011; 8:1161–1174. [PubMed: 21615213]
2. Swan EE, Mescher MJ, Sewell WF, et al. Inner ear drug delivery for auditory applications. *Adv Drug Deliv Rev.* 2008; 60:1583–1599. [PubMed: 18848590]
3. Schleiss MR, McVoy MA. Overview of congenitally and perinatally acquired cytomegalovirus infections: recent advances in antiviral therapy. *Expert Rev Anti Infect Ther.* 2004; 2:389–403. [PubMed: 15482204]
4. Ahmed A. Antiviral treatment of cytomegalovirus infection. *Infect Disord Drug Targets.* 2011; 11:475–503. [PubMed: 21827432]
5. Rivera T, Sanz L, Camarero G, Varela-Nieto I. Drug delivery to the inner ear: strategies and their therapeutic implications for sensorineural hearing loss. *Curr Drug Deliv.* 2012; 9:231–242. [PubMed: 22283653]
6. Barrs DM. Intratympanic corticosteroids for Meniere's disease and vertigo. *Otolaryngol Clin North Am.* 2004; 37:955–972. [PubMed: 15474104]
7. Barrs DM, Keyser JS, Stallworth C, McElveen JT Jr. Intratympanic steroid injections for intractable Meniere's disease. *Laryngoscope.* 2001; 111:2100–2104. [PubMed: 11802004]
8. Dodson KM, Woodson E, Sismanis A. Intratympanic steroid perfusion for the treatment of Meniere's disease: a retrospective study. *Ear Nose Throat J.* 2004; 83:394–398. [PubMed: 15266874]
9. Banerjee A, Parnes LS. Intratympanic corticosteroids for sudden idiopathic sensorineural hearing loss. *Otol Neurotol.* 2005; 26:878–881. [PubMed: 16151332]
10. Rauch SD. Intratympanic steroids for sensorineural hearing loss. *Otolaryngol Clin North Am.* 2004; 37:1061–1074. [PubMed: 15474111]
11. Shi J, Yang J, Wu H, et al. Intratympanic dexamethasone medication for sudden sensorineural hearing loss: clinical evaluation. *Lin Chuang Er Bi Yan Hou Ke Za Zhi.* 2006; 20:749–751. [PubMed: 17058923]
12. Bowe SN, Jacob A. Round window perfusion dynamics: implications for intracochlear therapy. *Curr Opin Otolaryngol Head Neck Surg.* 2010; 18:377–385. [PubMed: 20808222]
13. McCall AA, Swan EE, Borenstein JT, et al. Drug delivery for treatment of inner ear disease: current state of knowledge. *Ear Hear.* 2010; 31:156–165. [PubMed: 19952751]
14. Horie RT, Sakamoto T, Nakagawa T, et al. Sustained delivery of lidocaine into the cochlea using poly lactic/glycolic acid microparticles. *Laryngoscope.* 2010; 120:377–383. [PubMed: 19950377]
15. Kelly RM, Meyer JD, Matsuura JE, et al. In vitro release kinetics of gentamycin from a sodium hyaluronate gel delivery system suitable for the treatment of peripheral vestibular disease. *Drug Dev Ind Pharm.* 1999; 25:15–20. [PubMed: 10028414]
16. Paulson DP, Abuzaid W, Jiang H, et al. A novel controlled local drug delivery system for inner ear disease. *Laryngoscope.* 2008; 118:706–711. [PubMed: 18182968]

17. Piu F, Wang X, Fernandez R, et al. OTO-104: a sustained-release dexamethasone hydrogel for the treatment of otic disorders. *Otol Neurotol*. 2011; 32:171–179. [PubMed: 21099726]
18. Wang X, Dellamary L, Fernandez R, et al. Dose-dependent sustained release of dexamethasone in inner ear cochlear fluids using a novel local delivery approach. *Audiol Neurotol*. 2009; 14:393–401. [PubMed: 19923809]
19. Koizumi T, Higuchi WI. Analysis of data on drug release from emulsions. II. Pyridine release from water-in-oil emulsions as a function of pH. *J Pharm Sci*. 1968; 57:87–92. [PubMed: 5652140]
20. Flynn GL, Smith EW. Membrane diffusion. I. Design and testing of a new multifeatured diffusion cell. *J Pharm Sci*. 1971; 60:1713–1717. [PubMed: 5133927]
21. Kneczke M, Landersjo L, Lundgren P, Fuhrer C. In vitro release of salicylic acid from two different qualities of white petrolatum. *Acta Pharm Suec*. 1986; 23:193–204. [PubMed: 3799212]
22. Emami J. In vitro–in vivo correlation: from theory to applications. *J Pharm Pharm Sci*. 2006; 9:169–189. [PubMed: 16959187]
23. Qiao M, Chen D, Ma X, Liu Y. Injectable biodegradable temperature-responsive PLGA–PEG–PLGA copolymers: synthesis and effect of copolymer composition on the drug release from the copolymer-based hydrogels. *Int J Pharm*. 2005; 294:103–112. [PubMed: 15814234]
24. Azarmi S, Roa W, Lobenberg R. Current perspectives in dissolution testing of conventional and novel dosage forms. *Int J Pharm*. 2007; 328:12–21. [PubMed: 17084051]
25. Flynn GL, Shah VP, Tenjarla SN, et al. Assessment of value and applications of in vitro testing of topical dermatological drug products. *Pharm Res*. 1999; 16:1325–1330. [PubMed: 10496646]
26. Jeong B, Kim SW, Bae YH. Thermosensitive sol-gel reversible hydrogels. *Adv Drug Deliv Rev*. 2002; 54:37–51. [PubMed: 11755705]
27. Zentner GM, Rath R, Shih C, et al. Biodegradable block copolymers for delivery of proteins and water-insoluble drugs. *J Control Release*. 2001; 72:203–215. [PubMed: 11389999]
28. Goycoolea MV, Lundman L. Round window membrane. Structure function and permeability: a review. *Microsc Res Tech*. 1997; 36:201–211. [PubMed: 9080410]
29. Chelikh L, Teixeira M, Martin C, et al. High variability of perilymphatic entry of neutral molecules through the round window. *Acta Otolaryngol*. 2003; 123:199–202. [PubMed: 12701740]
30. Lundman L, Bagger-Sjoberg D, Holmquist L, Juhn S. Round window membrane permeability: An in vitro model. *Acta Otolaryngol Suppl*. 1989; 457:73–77. [PubMed: 2929337]
31. Salt AN, Plontke SK. Principles of local drug delivery to the inner ear. *Audiol Neurotol*. 2009; 14:350–360. [PubMed: 19923805]
32. Kanzaki S, Fujioka M, Yasuda A, et al. Novel in vivo imaging analysis of an inner ear drug delivery system in mice: comparison of inner ear drug concentrations over time after transtympanic and systemic injections. *PLoS One*. 2012; 7:e48480. [PubMed: 23251331]
33. Wang Y, Gao X, Kuriyavar S, et al. Incorporation, release, and effectiveness of dexamethasone in poly(lactic-co-glycolic acid) nanoparticles for inner ear drug delivery. *J Nanotechnol Eng Med*. 2011; 2:011013-1–011013-7.
34. Thaler M, Roy S, Fornara A, et al. Visualization and analysis of superparamagnetic iron oxide nanoparticles in the inner ear by light microscopy and energy filtered TEM. *Nanomedicine*. 2011; 7:360–369. [PubMed: 21146633]
35. Du X, Chen K, Kuriyavar S, et al. Magnetic targeted delivery of dexamethasone acetate across the round window membrane in guinea pigs. *Otol Neurotol*. 2013; 34:41–47. [PubMed: 23187928]
36. Mondalek FG, Zhang YY, Kropp B, et al. The permeability of SPION over an artificial three-layer membrane is enhanced by external magnetic field. *J Nanobiotechnology*. 2006; 4:4. [PubMed: 16603066]
37. Lee SH, Lee JE, Baek WY, Lim JO. Regional delivery of vancomycin using pluronic F-127 to inhibit methicillin resistant *Staphylococcus aureus* (MRSA) growth in chronic otitis media in vitro and in vivo. *J Control Release*. 2004; 96:1–7. [PubMed: 15063024]
38. Salt AN, Hartsock J, Plontke S, et al. Distribution of dexamethasone and preservation of inner ear function following intratympanic delivery of a gel-based formulation. *Audiol Neurotol*. 2011; 16:323–335. [PubMed: 21178339]

Figure 1. Schematic diagrams of (a) intratympanic injection of a formulation; the (b) conventional and (c) modified diffusion cell systems in the experiments; and the dimensions of the formulations in the donor chambers of the (d) conventional and (e) modified diffusion cell systems used in the computer simulations. Sizes and dimensions are not to scale.

Figure 2.

Cidofovir release profiles of four formulations and a reference control in the experiments with the conventional diffusion cells *in vitro*. PBS was the control. Data after 50 h are not shown. Symbols: 20% Pluronic (diamonds); 30% Pluronic (squares); 20% commercial PEG-PLGA-PEG (circles); 10% synthesized PEG-PLGA-PEG (triangles); PBS control (crosses). Mean $\pm$ SD ($n = 4$).

Figure 3.

Cidofovir release profiles of four formulations and the control in the experiments with the modified diffusion cells *in vitro*. PBS was the control. Symbols: 20% Pluronic (diamonds); 30% Pluronic (squares); 20% commercial PEG-PLGA-PEG (circles); 10% synthesized PEG-PLGA-PEG (triangles); PBS control (crosses). Mean $\pm$ SD ($n = 4$).

Figure 4.

Simulations of drug release profiles of the (a) conventional diffusion cells and (b) modified diffusion cells. (a) Diffusion cell dimensions: 2.5 mm radius × 2.5 mm height with diffusion opening of 2.5 mm radius and diffusion coefficient = $9.0 \times 10^{-6} \text{ cm}^2/\text{s}$ (solid line), diffusion coefficient = $4.5 \times 10^{-6} \text{ cm}^2/\text{s}$ (dotted line), and diffusion coefficient = $2.3 \times 10^{-6} \text{ cm}^2/\text{s}$ (dashed-dotted line). (b) Diffusion cell dimensions: 2.5 mm radius × 2.5 mm height with diffusion opening of 0.30 mm radius and diffusion coefficient = $9.0 \times 10^{-6} \text{ cm}^2/\text{s}$ (solid line); diffusion opening of 0.25 mm radius and diffusion coefficient = $9.0 \times 10^{-6} \text{ cm}^2/\text{s}$

(black dashed line); diffusion opening of 0.20 mm radius and diffusion coefficient = $9.0 \times 10^{-6} \text{ cm}^2/\text{s}$ (dashed-dotted line); diffusion opening of 0.25 mm radius and diffusion coefficient = $4.5 \times 10^{-6} \text{ cm}^2/\text{s}$ (gray dashed line).

Figure 5.

Representative semi-log plots of drug remaining in the donor chamber versus time for the computer simulation results of the (a) conventional diffusion cells and (b) modified diffusion cells. (a) Diffusion cell dimensions: 2.5 mm radius $\times$ 2.5 mm height with diffusion opening of 2.5 mm radius and diffusion coefficient = $9.0 \times 10^{-6} \text{ cm}^2/\text{s}$ (solid line), diffusion coefficient = $4.5 \times 10^{-6} \text{ cm}^2/\text{s}$ (dotted line), and diffusion coefficient = $2.3 \times 10^{-6} \text{ cm}^2/\text{s}$ (dashed-dotted line). (b) Diffusion cell dimensions: 2.5 mm radius $\times$ 2.5 mm height with diffusion opening of 0.30 mm radius (solid line), diffusion opening of 0.25 mm radius

(dashed line), and diffusion opening of 0.20 mm radius (dashed–dotted line) with diffusion coefficient = 9.0×10^{-6} cm²/s.

Figure 6.

Representative drug released versus square root of time plots of the (a) *in vitro* experimental results and (b) computer simulation data with the modified diffusion cells. (a) Formulations: 20% Pluronic (diamonds); 30% Pluronic (squares); 20% commercial PEG-PLGA-PEG (circles); 10% synthesized PEG-PLGA-PEG (triangles). (b) Diffusion cell dimensions: 2.5 mm radius $\times$ 2.5 mm height with diffusion opening of 0.30 mm radius (solid line), diffusion

opening of 0.25 mm radius (dashed line), and diffusion opening of 0.20 mm radius (dashed–dotted line) with diffusion coefficient = $9.0 \times 10^{-6} \text{ cm}^2/\text{s}$.

Table 1

Vehicles of the formulations used in this study and their transition temperatures.*

Vehicle	Composition (% w/w)	Transition temperature range (°C) [†]
Control	100% PBS	Not applicable
20% Pluronic	20% Pluronic, 80% water	19–22
30% Pluronic	30% Pluronic, 70% water	9–13
PLGA–PEG–PLGA (commercial), AK24	20% PLGA–PEG–PLGA, 80% PBS	34–35
PLGA–PEG–PLGA (synthesized)	10% PLGA–PEG–PLGA, 90% PBS	18–22

* Cidofovir was added to these vehicles to prepare the cidofovir formulations at final concentration of 5 mg/g (0.5%, w/w) cidofovir.

[†] The transition temperatures represent solution-to-gel transitions when temperature increases. Transition temperatures were determined without the drug.

Table 2

Release rate constants of the first-order release model of the formulations used in this study.*

Vehicle	–Rate constant (h⁻¹), regression analysis <i>R</i>², conventional diffusion cells[†]	–Rate constant (h⁻¹), regression analysis <i>R</i>², modified diffusion cells
Solution control	–1.45 [‡]	–0.46 ± 0.07, 0.954 [§]
20% Pluronic	–0.12 ± 0.03, 0.840	–0.033 ± 0.001, 0.999
30% Pluronic	–0.11 ± 0.02, 0.928	–0.030 ± 0.002, 0.982
PLGA–PEG–PLGA (commercial), AK24	–0.09 ± 0.01, 0.958	–0.013 ± 0.001, 0.991
PLGA–PEG–PLGA (synthesized)	–0.130 ± 0.003, 0.998	–0.0051 ± 0.0002, 0.993

* Means ± SE.

[†] Rate constants were determined using data up to 24 h due to complete drug release around 48 h.

[‡] Rate constant was estimated using data up to 4 h as complete drug release was achieved at that time.

[§] Rate constant was determined using data up to 8 h as drug release was almost complete at that time.