Dexamethasone (DXM)-Coated Poly(lactic-co-glycolic acid) (PLGA) Microneedles as an Improved Drug Delivery System for Intracochlear Biodegradable Devices

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Uniform drug delivery techniques are challenging to develop for the inner ear due to the complexity of the cochlear anatomy. A promising solution is the use of biodegradable polymers because the continuous release of therapeutics without introducing toxic compounds is desirable. Using a microneedle approach lends the polymeric microneedle the capability to be placed inside of the scala tympani, releasing drugs overtime. Poly(lactic-co-glycolic acid) (PLGA) microneedles is prepared by dissolving dimethyl sulfoxide with either a) Rhodamine B, to study the drug release profile in vitro, b) FM1-43, to study the drug release profile in vivo, or c) dexamethasone (DXM), to protect hair cell (HC) loss in vivo. The Rhodamine B studies show that the dye begins release from the microneedles within 30 min. The ototoxicity assessment of the DXM-coated microneedles in vitro shows a significant reduction of HC losses when compared to control microneedles in an ototoxic environment. In vivo data show reduced hearing threshold for animals treated with DXM-infused microneedles, providing a proof of concept of the methodology developed. Drug-infused polymeric microneedles provide a promising method to deliver DXM to the inner ear over controlled periods of time protecting hair cells, thus minimizing hearing loss (HL).

1. Introduction

The design and development of reliable, biocompatible, and effective drug delivery systems for both systemic and localized applications is paramount given the emergence of novel biopharmaceuticals, biologics, and other therapeutic agents that require tailored administration characteristics different from those afforded by traditional systems. One of the FDA approved biodegradable polymers, poly(lactic-co-glycolic acid) (PLGA), has aided in this endeavor due to its versatile use as a scaffold for incorporation of drugs and its ability to be employed as a controlled release delivery system. The versatility of its synthesis, degradation, and modification endows this copolymer with unique tunability making it an ideal drug delivery scaffold for a plethora of applications.[1–3] In that regard, a field where these PLGA polymers find application is in the delivery of hearing loss therapeutic agents. Hearing loss (HL) is the most prevalent sensory disability globally, with ≈465 million people in the world deaf or hearing impaired.[4] Inner ear disorders such as sudden sensorineural hearing loss (SNHL), Ménière’s disease, and hereditary hearing impairment severely affect patient’s quality of life, not only due to the direct challenge associated with reduced hearing, but also because of the fatigue, frustration, embarrassment, and social isolation many patients report.[5,6] In addition, HL represents an economical burden of nearly $750 billion related to healthcare costs and loss of work. It is estimated that 50% of hearing loss can be prevented, which showcases the opportunity to reduce if not eliminate this condition and its consequences.[7] Unfortunately, to date inner ear drug delivery techniques have fallen short of the requirements to combat inner ear hair cell (HC) death and thus, HL remains a largely unaddressed disability.[8] The underlying...
molecular mechanism of HC death in the organ of Corti (OC) is not yet fully elucidated. However, the cascade of events to create the electrical signal is known to be terminated, resulting in HL.[9] To address these issues, studies have repeatedly shown that dexamethasone (DXM), a corticosteroid, combats HC death by altering the expression levels of apoptosis related genes.[10–15] While systemic forms of drug delivery of DXM are available, undesirable side effects are common and the effective dose may not reach the ear when given systemically.[16] Other currently available treatment methodologies and strategies, apart from new drug development, include intratympanic (IT) injections of drugs, cochlear implants, stem cell therapies, manipulation of gene expression, viral vectors, thermosensitive gels, nanoparticles, and placement of drug delivery materials on the surface of the round window membrane (RWM), amongst others.[17–20] However, the development of these techniques for the delivery of DXM into the inner ear has been hindered by the complex cochlear anatomy, which limits molecular transportation of drugs.[17,21] In addition to the inherent complications caused by the cochlear anatomy, the short-lived time period that the perilymph solution experiences in the cochlea creates additional convolutions. Specifically, the blood labyrinth barrier separates the inner ear from systemic circulation with tight junctions, protecting the integrity of inner ear in a similar fashion to the blood brain barrier, and functions as a biochemical barrier by employing efflux pump systems to remove foreign agents from inner ear circulation, including DXM.[22] To overcome these challenges, a novel microfabricated drug delivery system was designed and developed that was implanted into the ear to deliver a uniform dose of the drug of choice over an extended period in a cost-effective manner. PLGA, a well-known biodegradable polymer, and DXM were employed as the materials for the fabrication of the microneedles. The polymeric microneedle implant has enough mechanical strength to pierce the RWM and be placed inside the cochlea, a procedure of which is well documented in the literature using cochlear implants.[23] Placing the microneedle directly inside of the scala tympani allows for the appropriate amount of drug to be released overtime directly to the affected area. Additionally, the extended-release profile of the polymer allows necessary doses of drug to remain in the perilymph solution despite perilymph clearance. Herein described is the method of microneedle development including fabrication of a customized mold. Furthermore, in vitro testing is described using OC explants exposed to said microneedles as well as different in vivo experimentation designed to provide a proof of concept as well as to study the preliminary intracochlear drug release profile through the assessment of HC viability and functionality. The developed technology not only addresses the hindrances associated with the convoluted anatomy and dynamic environment of the cochlea, but also proposes a new methodology that is applicable for many other intricate areas of the body that require targeted, localized, and uniform drug delivery.

2. Results and Discussion

2.1. Microneedle design for In Vitro experiments

The initial microneedle design for the in vitro experiments was on a larger scale for preliminary investigation. Different com-

positions of glycolic acid and lactic acid were investigated as the crystallinity, and therefore the mechanical strength of the PLGA, varies depending on the monomer ratios, from amorphous to fully crystalline. Polyactic acid contains methyl side chain groups that increase the disorder of the polymer chain depending on the ratio of lactic to glycolic acid. Additionally, by altering the ratio of lactic acid to glycolic acid, the degradation time changes due to the additional methyl group on the lactic acid affecting the hydrolysis of the polymer, making it less hydrophilic and slower to degrade.[24] Thus, the 50:50 PLGA copolymer was selected to manufacture the microneedles as this ratio of lactic acid to glycolic acid degrades the quickest, which was desirable for the encapsulated drug to reach and protect the HC of the cochlea accordingly. To fabricate the microneedles, a positive mold was 3D printed using a custom designed mold via CAD software. A negative mold was cast from the positive 3D printed mold using polydimethylsiloxane (PDMS) due to its low cost, high flexibility, and ease of use (Figure 1D).[25,26] The microneedles were then prepared by pipetting a homogenous solution of PLGA and DMSO into the mold and the solvent was allowed to evaporate overnight. The microneedle design incorporated a circular component on each end of the structure to allow for easier handling during the development process, which is subsequently removed to produce five microneedles per casting structure.

2.2. Biocompatibility Studies

Biocompatibility of the PLGA copolymer was investigated via analysis of 150 µm segments from the basal, middle, and apical turns of the OC’s basilar membrane as these areas are important for low-, middle-, and high-frequency hearing, respectively (Figure 2). Studies were then performed to investigate potential toxicity of DMSO, ethyl acetate (EtOAc), and acetone as the solvents for the dissolving of PLGA for physical manipulation. Microneedles were prepared using these solvents and the experiment was carried out as described in the Experimental Section. The percent hair cell loss was calculated for 150 µm segments of the basal, middle, and apical turns of the OCs exposed to DMSO and acetone, both of which showed minimal HC loss when compared with EtOAc (Figure 3). Since the preliminary in vitro studies using EtOAc showed hair cell loss, only DMSO and acetone were investigated with additional OC explants (n = 3). DMSO creates a homogenous solution when dissolved with other drugs relevant for future studies including other experimental drugs.
while acetone does not. Thus, DMSO was selected as the solvent of choice for the microneedle preparation for this application.

2.3. Drug Release Profile

After demonstrating that the material and solvent of the microneedles are safe and nontoxic, the microneedles were then cast using a fluorescent compound commonly used to characterize drug release profiles, Rhodamine B, in place of DXM to study the hypothetical drug migration in vitro in an artificial perilymph solution. While DXM and Rhodamine B are inherently different compounds, this study was performed as an initial proof of concept to demonstrate that PLGA can encapsulate a foreign compound uniformly and subsequently release said molecule upon hydrolysis in an aqueous environment. The release profile demonstrated that the PLGA copolymer begins release of Rhodamine B within 30 min of contact with the artificial perilymph solution. The initial release of the drug was rapid and began to slowly trend toward a more uniform release overtime (Figure 4). To reduce the lag time from microneedle placement to initial drug release, the subsequent set of microneedles for in vitro experiments included an additional experimental group with a coating of pure DXM on the surface of the microneedle to deliver an immediate bolus of drug upon insertion. This coating served as a bridge between microneedle insertion and DXM release.

Following the demonstration of the immediate release of Rhodamine B from the microneedles for preliminary investigation, the long-term release profile of the drug-infused microneedles was assessed via liquid chromatography with tandem mass spectrometry (LCMS/MS). A drug-infused microneedle was placed in an artificial perilymph solution at 37 °C and the concentration of drug in solution was measured every week for 1 month \((n = 4)\) (Figure 5). The concentration of drug released after 1 month was

![Figure 2](image-url)  
**Figure 2.** Confocal Images of organ of Corti organotypic cultures exposed only to PLGA beads demonstrating hair cell viability and thus biocompatibility of the polymer after 72 h of culture. Bars are 50 μm.

![Figure 3](image-url)  
**Figure 3.** % HC Loss of OC explants exposed to microneedles prepared using different solvents demonstrating the biocompatibility of DMSO and acetone and the ototoxicity of EtOAc. Green: number of HCs in the basal; orange: middle; red: apical turns of the organ of Corti.

![Figure 4](image-url)  
**Figure 4.** A) UV–vis spectra of the perilymph solution containing the Rhodamine B doped microneedle at different time points demonstrating release of the compound beginning after 30 min. B) Rhodamine B release profile from the microneedles providing a visual representation of the rapid initial release that plateaus to a continuous release overtime.
approximately one half of the total drug encapsulated in the microneedle, demonstrating the continued release of the drug from the microneedle over an extended period.

2.4. Safety and Otoprotective Properties

To investigate the safety and otoprotective properties of the microneedles, OC explants were exposed to different experimental conditions ($n = 6$ explants/group). Gentamicin (GM) was used to mimic an ototoxic environment leading to about 50% of HC loss without treatment (this ideal concentration of GM was determined in titration trials performed before the actual experiments). Group 1: GM only (positive control); group 2: GM and DXM in solution (75 $\mu$g mL$^{-1}$); group 3: GM and DXM-coated and infused microneedle (DXM coating); group 4: GM and DXM-infused microneedle (no DXM coating); and group 5: only media without GM, DXM or microneedles (negative control). HC counts were determined via confocal microscopic examination of phalloidin-FITC stained OC explants and plotted for each of the five groups. DXM-coated microneedles showed a significant reduction of both inner hair cell (IHC) and outer hair cell (OHC) losses when compared to the non-coated prototype ($p < 0.0001$). A similar efficacy in protecting from HC loss was found when compared to DXM solution after 72 h of culture (Figure 6). This was confirmed visually via confocal microscopy images (Figure 7). Thus, DXM-coated and infused microneedles protected both IHCs and OHCs, from the base to the apex of the cochlea, which encompasses all frequencies of hearing that can potentially cause ototoxicity, thus demonstrating the functionality of the developed technology in vitro.

2.5. Fabrication of Miniature Microneedles for In Vivo Models

To prepare the microneedles for insertion into the cochlea, a miniaturized mold was engineered via an SU-8 photolithography process in the Dr. JT Macdonald Foundation Biomedical Nanotechnology Institute at the University of Miami (BioN-IUM) Nanofabrication facility (Figure 8). The microneedle was designed with a length of 3.5 mm to sit in the base of the scala tympani (Figure 9). The average full-length dimensions of the rat scala tympani is 11 mm. This small parameter allows the microneedle to be inserted into the cochlea without fracture of the osseous spiral lamina, thus reducing modiolar injury.[29] The FM1-43FX microneedles were prepared by codissolving PLGA copolymer and FM1-43FX, a fixable fluorescent dye that behaves as a permanent blocker of the mechanotransducer channel, in DMSO (Figure 8D). The final concentration of the FM1-43FX dye was 1% (w/w) of the PLGA. For microneedles carrying DXM, 25 mg of 50:50 PLGA copolymer and 2.5 mg of DXM were dissolved in 100 $\mu$L of DMSO over 3 h at RT, and this homogenous solution was then cast into the PDMS mold. As each microneedle weighs $\approx 300 \mu$g, each microneedle contains 50 $\mu$g of DXM and 250 $\mu$g of PLGA, indicating the microneedle drug loading efficiency is 16.6%.

2.6. Physical Characterization of Polymeric Microneedles

The DXM polymeric microneedles were characterized for their mechanical properties and imaged to investigate the surface and integrity of the samples. Scanning electron microscopy (SEM) analysis revealed that the microneedles have a smooth outer surface, which allows for the microneedles to effortlessly pierce the RWM causing minimal insertion trauma.[30] The SEM imaging also confirmed that the microneedles have a length of 3.5 mm and diameter of 400 $\mu$m as designed, yielding a tip area of 1.26 $\times$ 10$^{-7}$ m$^2$ (Supplementary Information). The nanohardness ($H$) was measured to be $40.35 \pm 1.54$ MPa, reduced elastic modulus...
Figure 7. A–C) Basal turns of organotypic OCs exposed to an ototoxic environment (5 × 10^{-6} M GM) and different otoprotective measures. A) Control ototoxic environment showing HC loss; B) DXM-blended microneedle showing improved otoprotection; C) DXM-blended and coated microneedle demonstrating 100% HC viability and otoprotection. GM induced a significant HC loss that was more prominent in the IHCs compared to the OHCs. Bar is 50 × 10^{-6} m.

Figure 8. A) Mask of preliminary microneedle design; B,C) positive microneedle mold on silicon wafer; D) miniaturized FM1-43FX microneedles. Bars are 2.5 mm.

(Er) was 1.43 ± 0.06 GPa and the contact depth (hc) was 479.73 ± 10.04 nm (N = 5). The H threshold required to puncture the RWM of adult guinea pigs has been found to be 1.19 mN, which translates to 9.47 × 10^{-3} MPa over the area of the designed microneedle tip, with the force required to puncture a human RWM being four to five times this value. The measured H value of 40.35 ± 1.54 MPa demonstrated that the microneedles have over ≈4000 × the mechanical strength required to puncture rodent RWMs, as well as ample force for use in human studies for future work.

2.7. In Vivo Analysis of the Polymeric Microneedles

To study the impact the drug-infused microneedles have on combating inner ear HC loss, two in vivo studies were performed. For study 1, FM1-43FX dye infused microneedles were implanted into the scala tympani of male C57BL/6 mice (n = 3) via a retroauricular approach for 3–7 days. At this time, cochleae were harvested and processed for microscopic visualization to determine the distribution of the dye along the cochlea’s HCs. Oto-toxicity was intentionally not used, since intact HCs are necessary for entry of the FM1-43FX dye. For study 2, Norway-Brown rats were used instead of mice due to the larger average dimensions of the basal turn of the rat scala tympani when compared with mice. Study 2 intended to determine the protective effects of the drug-infused microneedle against HL induced by introduction of a foreign object to the ear. For this study, animals were randomly divided into two groups (n = 3 per group): group 1: no ototoxicity with PLGA microneedle as a positive control, and group 2: no ototoxicity with DXM-infused microneedle. Subsequently, animals underwent hearing tests (see below) to assess functional
2.8. In Vivo Analysis Demonstrating Drug Release and Diffusion within the Cochlea

The miniaturized FM1-43FX-infused microneedles were implanted in the scala tympani of adult C57BL/6 mice for 1 week which confirmed slow release of the dye from the microneedles. A red signal was identified in the HCs at different levels of the cochlea suggesting that the dye had passed through the mechanotransduction apparatus, confirming the diffusion of the dye through the perilymph from the basal turn all the way to the apex of the cochlea (Figure 11).

2.9. In Vivo Feasibility Demonstration of the Functionality of the Polymeric Microneedles

Following successful demonstration of the migration of the FM1-43 dye to the HCs in vivo, further in vivo investigation was performed following the same procedure to demonstrate the feasibility of the proposed platform with DXM using microneedles with and without the drug.

Auditory brainstem response (ABR) data was collected to determine the hearing threshold of the animals throughout the experiment. Specifically, baseline ABR was recorded on day 0, the microneedle was inserted on day 1, and ABR data was collected 1 day, 3 days, and 1 week post insertion. This timeline was selected as previous studies using the same animal model and approach have shown significant differences between threshold ABR data and ABR data collected 7 days post cochlear implantation into the scala tympani through the RWM.[34] ABR data demonstrated that hearing thresholds of animals were lower on days 2–7 for each frequency analyzed in ears implanted with a DXM-coated and DXM-infused PLGA microneedle when compared to the group which received a control PLGA microneedle (Figure 12). An ABR hearing threshold of only 5–6 dB is the point of intensity in which an adult can detect the presence of a stimulus.[35] Thus, the ABR data from Figure 11 demonstrate reduced hearing thresholds across all frequencies of more than 10 dB on day 7, a reduction of which is large enough to affect hearing. This lower threshold reveals that DXM is not only being released from the microneedle but is also reaching the appropriate HCs to effectively allow the animals to hear at lower levels consistently across all frequencies. The ABR readings are anticipated to return to baseline as the confocal microscopy of both groups of in vivo animals showed no hair cell death in the basal, middle, and apical turns of the cochlea indicating no HL associated with cochlear insertion trauma (Figure 13). This in vivo data demonstrates the functionality of the microneedle releasing DXM in real time over an extended period without introducing any undesirable side effects while improving hearing thresholds as a proof of concept for the developed platform. Extended in vivo analysis is currently being conducted to determine the long term otoprotective effects of the DXM microneedle with a larger sample size over an extended period of time.

3. Conclusion

There is need for novel drug delivery systems to treat complex conditions in the human body, especially those related to otologic disorders. An ideal drug delivery system for such applications should need minimal number of drug doses, injections, or insertions, depending on the approach, as well as have minimal side effects. While there are no FDA approved drugs developed specifically to treat inner ear disease, approved drugs are used that provide systematic relief.[36] Previous and current methods used to treat HL include systemic administration, IT injections, cochlear implants, nanoparticle formulations and various other methodologies that place drug delivery materials on the RWM directly for osmotic delivery. Systemic delivery often fails to deliver the required amount of drug to the inner ear due to the blood labyrinth barrier, which simultaneously requires higher doses of these drugs to cross. Unwanted side effects such as
irritability, hypertension, and organ damage are introduced after long term use of these drugs at high levels.⁶ IT injections were introduced to the field to combat the downfalls of systemic delivery systems. However, IT injections deliver significantly smaller amounts of drug to the inner ear due to minimal diffusion through the RWM and drainage of the drugs out of the ear via the Eustachian tube.¹² Additionally, the pharmacokinetic profile of the drugs administered intratympanically to the inner ear are highly dependent on the physiochemical property of the drug itself.¹⁷ Drug delivery materials were subsequently developed which were placed on or near the RWM, yet when these materials are displaced, the drug delivery is compromised. Furthermore, drugs with high molecular weight and low lipid solubility cannot traverse the RWM readily, even when directly applied onto the RWM.³⁸ A method to circumvent this issue was the introduction of polymeric based nanoparticles that are able to permeate the RWM readily, even when directly applied onto the RWM.³⁸ Most recently, it has been demonstrated that DXM-coated silicone rods are able to release DXM over extended periods of time into the inner ear in vivo.⁴¹ These data support our current claims that releasing DXM long term into the cochlea is beneficial, on the downside the drug coated silicone rods employed by Liebau et al. are not biodegradable and remain in the cavity, which may lead to foreign body response, immunotoxicity, and other long-term undesirable side effects. In our work, we demonstrated a solution to the various problems of the prior work described in the literature by incorporating the corticosteroid DXM, the anti-inflammatory drug of choice for treatment of conditions leading to HL, in a controlled delivery system that addresses the drawbacks associated with current delivery techniques such as minimal drug retention over extended periods of time as well as biodegradability, leaving no foreign bodies behind in the cochlea once the microneedle has fully dissolved. The biocompatible FDA approved polymer, PLGA, was chosen as the vehicle of choice due to its tunable degradation and mechanical characteristics. The 50:50 PLGA copolymer was selected, specifically, as this copolymer has equal amounts of lactic acid to glycolic acid, which has previously been shown to degrade the fastest.⁴² DXM was chosen as the drug of choice to minimize HL due to extensive prior investigation. However, the microneedle development was designed to be compatible with other common drugs used for HL including mannitol and L-N-acetylcysteine (L-NAC) to allow for a wider future applicability.⁴³ The PLGA microneedle is designed to pierce the RWM, which spontaneously heals itself, in order to enter the cochlea. Due to the mechanical strength of the polymer, the microneedle can be introduced into the cochlea without the use of any additional tools and without the introduction of any toxic agents as was demonstrated through the in vivo ototoxicity studies. Once the biocompatibility of the PLGA polymer was confirmed, further in vitro investigation demonstrated that a necessary coating of DXM was required to protect 85%, 87%, and 100% of the OHCs in the basal, middle, and apical turns of the cochlea, respectively, and 100% of the IHCs. In summary, the proposed work addresses an unmet need in the field of continuous drug delivery and more specifically in Otolaryngology, by employing microengineering that will decrease and/or prevent HL and, thus, enhance the quality of life of many patients who suffer from otologic disorders with the

**Figure 11.** FM1-43 blended microneedles implanted in adult mice for 1 week to confirm dye release and distribution through the perilymph. A,B) Control adult mice cochleae; C,D) mice adult cochleae exposed to a dye blended microneedle. A–D) 40× images using confocal microscopy; E) 63× magnification of sample. The dye is being slowly released from the microneedles and reaches HCs passing through the mechanotransduction apparatus of the HC, confirming the diffusion of the dye through the perilymph.
potential to lead to HL. This study provides useful insight into a promising tool that could potentially revolutionize the treatment of different debilitating conditions, especially in otology.

4. Experimental Section

Microneedle Fabrication: A custom positive mold was designed using Rhino 3D software (ver. 6; Rhinoceros Inc., Seattle, WA) and manufactured by employing a 3D printer (LulzBot TAZ5; Aleph Objects, Inc., Loveland, CO) using acrylonitrile butadiene styrene (ABS) (Filabot ABS 3mm Orange; Filabot, Inc., Barre, VT), a thermoplastic polymer, as the substrate (Figure 1A–C). The ABS mold was treated with acetone vapor in a thin layer chromatography (TLC) chamber for 30 min to smooth the surface of the plastic. To create the PDMS mold, a silicone elastomer base was mixed in a 1:10 ratio with silicone elastomer curing agent using a Sylgard 184 Silicone Elastomer Kit (Dow Corning Co., Midland, MI). Once homogenous, the mixture was poured into the positive 3D-printed mold, placed under vacuum (BestValueVac, Inc., Naperville, IL) until all air bubbles were
removed and left to cure overnight at room temperature (RT). The mold was placed at 65 °C for 2 h the following day to eliminate any sticky residue and then removed from the positive 3D-printed mold manually with a spatula. The microneedles were prepared by pipetting a homogenous solution of 1:4 (w/w) 50:50 PLGA copolymer (Sigma Aldrich, St Louis, MO) dissolved in DMEM (VWR International Co., Radnor, PA) into the custom PDMS mold. The mold was left overnight at 65 °C to allow the DMSO to evaporate, and the procedure was repeated until the mold was filled with PLGA copolymer. The microneedles were brought to RT and removed from the mold manually using the end circular structure, and the five hardend microneedles were cut from the excess material (Figure 1E). For microneedles carrying DXM, 25 mg of 50:50 PLGA copolymer and 2.5 mg of DXM were dissolved in 100 μL of DMSO over 3 h at RT, and this homogenous solution was then cast into the PDMS mold and incubated overnight at 65 °C to allow DMSO to evaporate before use. For the coated microneedles group, the microneedles were prepared by dipping them into a 10% DXM solution using DMSO as the solvent and allowed to dry for an additional 24 h in a desiccator before usage.

**Animals for Data Collection:** All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Miami (protocol # 17-225) and were in full compliance with published National Institute of Health (NIH) guidelines for the care and use of laboratory animals (guide for the care and use of laboratory animals, 8th edition, The National Academies Press, Washington, DC, 2011). All animals were purchased from Charles River Laboratories, Wilmington, MA, USA and kept on a 12 h light/dark cycle and fed a standard diet upon arrival. For the in vitro studies, OC explants were obtained from 3-day-old Sprague-Dawley rat pups by euthanizing the animals and extracting whole temporal bones (n = 12). The whole OC explants were dissected en bloc, and one OC explant per well was placed into 24-well culture plates in 400 μL of complete serum-free media consisting of Dulbecco’s modified Eagle’s medium (DMEM) supplemented with glucose (final conc. 6 g/L) and one OC explant per well was placed into 24-well culture plates in 400 μL of DMSO over 3 h at RT, and this homogenous solution was then cast into the PDMS mold and incubated overnight at 65 °C to allow DMSO to evaporate before use. For the coated microneedles group, the microneedles were prepared by dipping them into a 10% DXM solution using DMSO as the solvent and allowed to dry for an additional 24 h in a desiccator before usage.

**Drug Release Profile Study with Rhodamine B**

**Drug Release Profile Study with Rhodamine B:** A fluorescent compound, Rhodamine B, was used in place of DXM to study the drug release profile in an artificial perilymph solution. The Rhodamine B microneedles were prepared by dissolving PLGA copolymer and Rhodamine B in DMSO and the solution was cast into the PDMS mold. The final concentration of the Rhodamine B dye was 1% (w/w) of the PLGA. The prepared needles were then placed in a quartz cuvette containing an artificial perilymph solution described by Salt et al.[44] The absorbance of the perilymph solution was measured every 30 min using a spectrophotometer until the absorbance of the solution at 552 nm was constant.

**Extended Drug Release Profile Study with Dexamethasone:** One DXM-infused microneedle structure per sample was placed into 100 μL of an artificial perilymph solution at 37 °C. A sample was collected after 1, 2, 3, and 4 weeks for investigation of DXM concentration via LCMS–MS according to Bird and co-workers.[45] The obtained data was analyzed to determine the DXM concentration release per microneedle structure. This calculated data was then fitted to a variable slope non-linear sigmoidal dose–response curve using GraphPad Prism (GraphPad Software, La Jolla, California USA) to generate the calibration curve for the DXM concentration release per microneedle structure.

**In Vitro Investigation:** OC explants were dissected from 3-day-old Sprague–Dawley rat pups by inducing hypothermia and then extracting whole temporal bones (n = 30 explants). One OC explant per well was placed into 24-well culture plates in 400 μL of complete serum-free media with or without 5 × 10^{-8} M of Gentamicin (GM) to mimic an ototoxic environment leading to about 50% of HC loss without treatment (this ideal concentration of GM was determined in titration trials performed before the actual experiments). OC explants were exposed to different experimental conditions (n = 6 explants/group): group 1: GM only; group 2: GM and DXM in solution (75 μg mL^{-1}); group 3: GM and DXM releasing needle (no DXM coating); group 4: GM and DXM releasing needle (DXM coating); and group 5: only media without GM, DXM or needles. For groups 2–4, GM was added to the media containing OCs 30 min before adding the drug or needle. This allowed sufficient time for the GM to diffuse to the HCs. The microneedles were placed at 0.5–1 mm from the OC explant and the plates were cultured for 72 h.

**Microneedle Preparation for In Vivo Investigation:** To prepare the positive mold for the miniature microneedles, a silicon wafer was cleaned with ethanol, prebaked to remove residual moisture, coated with 100 μm thick SU-8 photosist using CEE 200XP Precision spin coater ( Brewer Science, Inc., Rolla, MO) and soft baked to remove any bubbles created during the coating process. UV light was applied via an OAI Model 804 MBA Optical Mask Aligner (OAI, San Jose, CA) through a custom-made soda lime mask onto the wafer and the wafer was post-baked, developed, and hard baked again to harden the final elevated pattern (Figure 2A–C). PDMS was then cast onto the wafer containing the desired pattern to create the corresponding negative mold.

**Physical Characterization of Polymeric Microneedles:** The DXM polymeric microneedles were characterized for their mechanical properties and imaged using a JOEL IT300 Ultrahigh Resolution Field Emission Scanning Electron Microscope (JOEL USA, Inc., Peabody, MA) to investigate the surface and integrity of the samples. SEM analysis revealed the microneedles have a smooth outer surface, which allows the microneedles to effortlessly pierce the RWM with minimal insertion trauma.[30] The SEM imaging also confirmed the microneedles to have a length of 3.5 mm and diameter of 400 μm as designed, yielding a tip area of 1.26 × 10^{-3} m^{2} (Supplementary Information). The nanohardness, H, and reduced elastic modulus, E_r, were obtained by performing nanoindentation tests using a Hysitron Tribolindenter Nanomechanical Test System (Bruker, Billerica, MA). A load of 250 μN was applied to the sample at 22 °C in a chamber with 35% relative humidity using a Diamond Berkovich indenter tip. The area of the residual indentation was measured and the nanohardness, H, was defined as the maximum load, P_{\text{max}}, divided by the residual indentation area, A_i, as seen in Equation (1).

\[ H = \frac{P_{\text{max}}}{A_i} \]  

Throughout the indentation process, the depth of penetration was recorded in relation to the load at that time. A load-displacement curve was generated, the slope of which indicated the stiffness, S, of the contact. The reduced elastic modulus, E_r, was then calculated using Equation (2), where A_r(h) is the area of the indentation at the contact depth, h_c, and β is a geometrical constant.

\[ E_r = \frac{1}{\beta} \frac{S}{\sqrt{A_r(h_c)}} \]  

The nanohardness (H) was measured to be 40.35 ± 1.54 MPa, reduced elastic modulus (E_r) of 1.43 ± 0.06 GPa and the contact depth (h_c) of 479.73 ± 10.04 nm (N = 5). The H threshold required to puncture the RWM of adult guinea pigs has been found to be 1.19 mN, which translates to 9.47 × 10^{-1} MPa over the area of the designed microneedle tip, with the force required to puncture a human RWM being four to five times this value.[13,12] The measured H value of 40.35 ± 1.54 MPa demonstrated the microneedles have over 4000x the mechanical strength required to puncture rodent RWMs, as well as ample force for use in human studies for future work.[31]
Animal Surgery for In Vivo Experiments: For microneedle placement, mice were anesthetized with a ketamine (90 mg kg\(^{-1}\))–xylazine (5 mg kg\(^{-1}\)) cocktail and rats were anesthetized with a ketamine (40 mg kg\(^{-1}\))–xylazine (5 mg kg\(^{-1}\)) cocktail. Buprenorphine (0.05 mg kg\(^{-1}\)) was administered directly following the procedure as well as 1-day post procedure. Standard guideline to assess the depth of the anesthesia was followed. Subsequently, antisepsis of the ears was achieved with topical iodine application, then subcutaneous 1% lidocaine was used for topical anesthesia. A retroauricular approach was used to expose the posterior bony shelf of the bullae, which was then opened to find the stapedial artery and the RWM right above it. This membrane was pierced by insertion of the microneedle into the scala tympani and a piece of fascia was used for final coverage to prevent any perilymphatic leakage during healing (Figure 4, video of procedure included in the Supplementary Information). Microneedle insertion technique through the RWM and final position in the scala tympani mirrors that of cochlear implants, the procedures and location of which are well documented in the literature. The microneedle dimensions were designed to rest in the base of the cochlea without migration due to the natural curve of the organ. Additionally, the bony bulla provides additional protection to ensure no outside forces move the microneedle. Closure of the surgical field was achieved in layers using a 5–0 silk suture followed by placement of the animals in a cage fitted with water circulating warming pad until fully awake, at which time the animals were returned to the division of veterinary resources (DVR) housing case. To measure the effect the microneedle had on vestibular function, the rats were monitored for head tilt, circling in the cage, and nystagmus. Animals were euthanized 7 days after microneedle insertion for cochlear harvesting and histological analysis.

Auditory Brainstem Response (ABRs): The hearing of both ears of each rat was assessed by ABR responses to pure-tone stimuli (1, 4, 8, and 16 kHz), the frequencies of which were selected based on previous studies.\(^1\) Recording electrodes were placed on the vertex (+) and superior post auricular areas (−) of the scalp, as well as an inferior limb (ground electrode), to obtain the ABR data. Responses were recorded using Intelligent Hearing Systems (IHS Smart EP, Miami, FL, USA) hardware and software. An Emyetomic Research ER2 insert earphone (Emyetomic Research, Elk Grove Village, IL, USA) was used to deliver the stimuli at a rate of 29 Hz via a custom designed silicone ear tip that was inserted into the rat’s ear canal. These responses were intensified via an Opti-Amp bioamplifier from IHS that was directly connected to the Smart EP system. The stimulation intensity was decreased in decrements of 10 dB until no ABR response was identifiable to determine the threshold values. Threshold values were confirmed visually by an investigator blinded to the study groups.

Histological Analysis: For histological analysis, culture media was washed out of OC explants and fixed with 4% paraformaldehyde (PFA) for 15–20 min before staining the HCs. Cochlear tissue from adult animals was harvested, fixed with 4% PFA overnight and then decalified with a solution of 10% ethylenediaminetetraacetic acid (EDTA) for 1 week while on a rocker. Subsequently, the OC were dissected out of the bony shelf and cochlear turns (e.g., base, middle, and apex) and were divided with diamond scissors under a dissecting microscope. To stain HCs, OC explants and adult tissues were washed with PBS, blocked with a solution of normal goat serum and 0.3% Triton X-100 (Fluka, St Louis, MO) for 1 h, permeabilized, incubated in phalloidin-fluorescein isothiocyanate (FITC) and 4',6-diamidino-2-phenylindole (DAPI) solution (Sigma Aldrich) for 45 min at RT to stain hair bundles, cuticular plates, and nuclei. Lastly, the stained OC specimens were washed in PBS, mounted on a glass slide with antifade mounting medium, cover-slipped, and observed with a confocal Zeiss Axiovert 700 microscope (Carl Zeiss AG, Jena, Germany). To analyze HC counts from OC explants or adult cochlea, 150 μm segments of each turn were used to count the number of IHCs and OHCs, respectively. Confocal microscopy provided a high-quality image and allowed for z-stacking of multiple images to analyze the cells of interest at various depths after mounting on the slide.

Statistical Analysis: To evaluate the statistical significance of HC counts and ABRs, ANOVA with Bonferroni post hoc testing was used. Statistical significance was set at \(p < 0.0001\) and \(p < 0.05\), respectively.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements
The authors would like to thank Dr. Kevin Luongo for experiment consultation and assistance with photolithography and Dr. Sung Jin Kim for SEM imaging. S.D. would like to thank the University of Miami Miller School of Medicine for the Lucille P. Markey Chair in Biochemistry and Molecular Biology. In addition, the authors are thankful to the Dr. JT Macdonald Foundation Biomedical Nanotechnology Institute for providing access to its Nanofabrication Facility and resources needed for the microfabrication work. The back cover of volume 4 issue 11 was created with BioRender.

Conflict of Interest
The authors declare no conflict of interest.

Authors contributions
D.C.P. assisted in investigation, problem solving, visualization, software, and writing original draft; S.G. assisted in investigation, problem solving, formal analysis, and writing, reviewing and editing; E.B. assisted in validation; E.D. assisted in methodology and visualization; S.K.D. assisted in project administration, problem solving, and editing; S.D. assisted in supervision, conceptualization, funding acquisition, resources, problem solving, and editing; F.T. assisted in supervision, conceptualization, funding acquisition and resources.

Data Availability Statement
Data available on request from the authors.

Keywords
cochlear implantation, drug delivery technology, microneedle, poly(lactic-glycolic acid), rat model

Received: July 9, 2021
Published online: August 8, 2021

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