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Silk Protein Bioresorbable, Drug-Eluting Ear Tubes: *Proof of Concept*

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Abstract

Otitis media with effusion (OEM) is a common pediatric pathology treated with topical fluoroquinolones (ear drops) and tympanoplasty tube, also referred to as ear tube, implantation for middle ear drainage. Commercially available ear tubes are fabricated using poly (lactic-co-glycolic acid) (PLGA) synthetic materials that are associated with long-complications due to premature extrusion. Resorbable materials have emerged as desirable alternatives to reduce extrusion-related complications, but often limited by fast resorption rates. Therefore, resorbable tubes with long-term functional integrity are required for future clinical translation. In this communication, a proof-of-concept study is reported on a bioresorbable and drug-eluting silk ear tube device. Preliminary in vitro assessments reveal time-dependent drug elution and anti-microbial properties, while maintaining long-term functional integrity in vivo. This report provides evidence of a silk ear tube with potential for future clinical translation and OEM treatment.

Graphical Abstract

Antimicrobial resorbable silk ear tubes are developed for treatment of pediatric otitis media with effusion (OEM). Silk tubes provide on-site dual delivery of active ingredients found in prescribed ear drops, along with device fabrication, biocompatibility and degradability. Proof-of-concept study reveals potential for silk material as an alternative to commercially available synthetic ear tubes.

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Keywords

OEM; tympanoplasty tube; bioresorbable; drug-eluting; silk fibroin

Introduction

Otitis media with effusion (OEM) is a common pediatric pathology leading to about 667,000 Tympanostomy tube (T-tube, or ear tube) implantations annually [1]. Eustachian tube (ET) dysfunction leads to improper middle ear drainage and onset of OEM, requiring temporary ear tube placement in the tympanic membrane (TM) for treatment [1]. Commercially available tubes such as the Shepard Teflon grommet, Armstrong beveled tube, Reuter-Bobbin tube and Goode T-tube are designed to treat for 1–2 years and then self-extrude [2].

However, this extrusion is often unpredictable and premature or prolonged TM presence can lead to subsequent surgical procedures or severe long-term complications, including hearing loss [2,3]. Resorbable materials are a desirable alternative to eliminate extrusion-related complications by resorbing and restoring TM tissue. Synthetic polymers, such as poly (lactic-co-glycolic acid) (PLGA) and poly (L-lactide) (PLA), are the earliest versions of resorbable ear tubes, but degradation through acid hydrolysis can lead to inflammation [4]. More recently, biocompatible hydrogels fabricated with hyaluronic acid (HA) and polyethylene glycol (PEG) diacrylate cross-links have been reported. Although this material improves biocompatibility, reported resorption after 1 month is insufficient for OEM treatment [3a].

There remains a need for a biocompatible and bioresorbable ear tube design that meets clinical requirements and incorporates novel antimicrobial drug-eluting technology. To address this need, we use silk fibroin protein to generate degradable and drug-eluting ear tubes. Silk fibroin possesses biocompatibility, tunable mechanics, degradability and FDA approval for some medical products [5]. Silk fibroin has been previously shown to be a useful reservoir for tunable drug release in a breadth of material formats; therefore we incorporated this approach for drug-eluting technology for ear tubes [6]. This work adopts an aqueous process of concentrated silk solutions to render a collar button drug-coated ear tube format and efficacy was assessed in vitro and in vivo. The goal of this study was to provide a proof-of-concept study for silk ear tubes with efficacious design features of resorbability and drug eluting capability that would improve future treatment of OEM.

Experimental

Silk Fibroin Solution:

Silk fibroin, hereafter referred to as silk, aqueous solutions were prepared using previously established protocols [5]. Briefly, 10 grams of *B. mori* silkworm cocoons were extracted for 30 minutes (min) in a 0.02M Na₂CO₃ (Sigma-Aldrich, St. Louis, MO) aqueous solution and rinsed three times for 20 min to remove sericin. The degummed cocoons were dried in a fume hood for more than 24 hours (h) and then dissolved in a 9.3 M LiBr (Sigma-St. Louis, MO) solution for 3–5 h in 60 °C. The solution was dialyzed for 2 days against distilled water using regenerated cellulose membranes (3,500 MWCO, Thermo Scientific, Rockford, IL). The solubilized protein solution was then centrifuged twice (9,700 RPM, 20 min, 4 °C) and the concentration was determined by placing a wet volume of solution in a 60 °C oven to measure the final dry weight, where the final solution was between 5–7 w/v %.

Ear Tube Fabrication:

Silk solution was loaded into cellulose dialysis tubing (3500 MWCO, Thermo Scientific, Rockford, IL) for 48 hours in a fume hood to increase the concentration to 10–12 w/v %. Silk was placed in a CentriVap concentrator (Labconco, Kansas City, MO) to achieve a final concentration of 20–25 w/v %. Cylindrical wax molds (Machinablewax.com, Inc., Traverse City, MI) with 6 wells, 3.30 cm in height and 0.76 cm diameter were parafilm sealed to a 1mm thick wafer bottom (0.76 diameter) (Sigma-Aldrich, St. Louis, MO). Concentrated silk solution was loaded into wax mold wells and submerged in 100 v/v % methanol for 24 h to render insoluble silk gel cylinders. Cylinders were ejected with a plastic punch, dried for 1 h at room temperature. To prevent warping, cylinders were placed back into respective wells and dried in a fume hood for 48 h. Final silk cylinders were machined into desired ear tube dimensions and measured with scanning electron microscopy (SEM) and ImageJ analysis. Non-drug coated tubes were autoclaved with a dry cycle of 25/15 min for steam and drying. Ethylene oxide (ETO) sterilization for drug-coated tubes was achieved with 100 % ETO atmosphere at 55 °C for 3 h and placed in a hood for 12 h pre-implantation.

Drug-coated Tubes:

Tubes were coated in-line with prescribed medications and incorporated ciprofloxacin hydrochloride and dexamethasone (Sigma-Aldrich, St. Louis, MO) at concentrations of 9 mg/mL and 3 mg/mL of silk solution. Drug was added to a 5 w/v % silk solution and mixed. Machined tubes were placed on 0.9 mm outer diameter (OD) Teflon rods (McMaster-Carr, Elmhurst, IL) and 100 µl of coating was applied with a detailing paintbrush. The coated tubes were placed in a methanol bath containing ciprofloxacin HCL and dexamethasone. Coated tubes were cross-linked in a methanol bath saturated with 2.7 mg/mL ciprofloxacin HCL and 35 mg/mL dexamethasone to eliminate solvent solubilization of coated drug. Tubes were placed on Teflon rods in a fume hood to dry for 24 h.

Tube Resorption:

Following previously reported methods, in vitro enzymatic resorption was examined for 16 days and tubes were incubated in 2 U/mL protease XIV, or PBS controls [7]. Residual water

was removed via incubation at 60 °C for 10 min prior to weight measurement. At day 16, tubes were dried at 60 °C for 24 h and final weight was recorded. In vivo resorption was determined by percent mass remaining after 8 weeks (Equation 1). At termination, tubes were placed in a 75 unit/mL collagenase type I from *Clostridium histolyticum* solution, incubated at 37 °C on a rocker (10 Hz, 90 min) to digest adhered TM tissue, then rinsed in DI water and dried at 37 °C for 24h to determine final weight. Collagenase concentration and digestion time was designed to only impact TM tissue, but not the silk [8a].

$$\text{Mass Remaining (\%)} = \left(\frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \right) * 100 \quad \text{Equation 1}$$

In vitro Drug Elution:

To assess antimicrobial properties, drug and control non-coated ear tubes were placed in 1 mL of lysogeny broth (LB) (Sigma-Aldrich, St. Louis, MO) containing 5.21×10^7 colony forming units (CFU)/mL of gram negative bacteria, *Moraxella catarrhalis* (ATCC, Manassas, VA), and incubated at 37 °C for 24 h. Post-24 hour incubation, opacity is compared to determine antimicrobial properties. To test efficacy at both killing and preventing bacterial growth, supernatant from the 24 h culture was plated on LB agar plates and incubated an additional 24 h at 37 °C.

For time-dependent drug release, tubes were incubated in 1mL PBS at 37 °C and supernatant was collected at 1, 3, 5, 10 h, and 1, 2, 3, 5, 6 and 7 days for high performance liquid chromatography (HPLC) quantification. HPLC was performed using previously established methods for ciprofloxacin HCl and dexamethasone quantification [8b]. The mobile phase was prepared with methanol, water, and triethylamine (60:39.5:0.5, v/v/v) and pH was adjusted to 3.0 ± 0.05 with orthophosphoric acid. The flow rate was set to 0.75 mL/min with an injection volume of 20 µL and detection wavelengths set to 254 and 241 nm for ciprofloxacin and dexamethasone. Reverse phase C18 column maintained at 25 °C was used for separation (Agilent ZORBAX Eclipse XDB C18, 5 µm particle size, L × I.D. 15 cm × 4.6 mm, Santa Clara, CA). Standard solutions were diluted with the mobile phase to prepare concentrations ranging 125 – 0.5 µg/mL.

Animal Preparation:

All animal experiments were conducted in accordance with a protocol approved by the Animal Care and Use Committee of the Massachusetts Eye and Ear Infirmary. Data were obtained from chinchillas (*Chinchilla lanigera*) with weights of 400 and 800 g. For each animal, a tube was placed in one ear through myringotomy incision and the contralateral ear served as a control with no implantation. Induction of anesthesia was performed with 25 mg/kg intramuscular ketamine, 3 mg/kg intramuscular xylazine and 0.02 mg/kg intramuscular atropine. The chinchilla's response to toe pinch was noted every 5–10 minutes while under anesthesia and if additional sedation was required, a maintenance dose of intramuscular ketamine at a dose of 12.5 mg/kg was administered. Rectal temperature was monitored and maintained at 39 °C by means of a servo-controlled battery-powered electrical heating pad. Heart and respiratory rate were monitored throughout the procedure.

Final examination of ear canals was performed using the 30 degree endoscope. The animal was anesthetized for terminal DPOAE and ABR measurements. Intraperitoneal fatal plus solution was administered at termination and bilateral TM's were dissected for tube removal.

Statistics

Unless otherwise noted, two-way analysis of variance (ANOVA) and Tukey post-hoc to test statistical significance was performed in GraphPad Prism Software v7.0 (GraphPad Software, San Diego, USA). P values < 0.05 were determined as statistically significant and reported as **** P < 0.0001, *** P < 0.0005, ** P < 0.005, * P < 0.05. All data are presented as mean $\pm$ standard deviation.

Results and Discussion

Ear Tube Fabrication:

Ear tubes were machined to clinically relevant size with outer collar diameter of 1.36 mm, inner collar diameter of 1.1 mm, collar-to-collar length of 1.7 mm and total tube length of 2.3 mm (Figure 1). Tube placement on Teflon rods for drug-coating methods prevented inner lumen occlusion during fabrication (Figure 1B). The fabrication method reported is highly tunable- machining alters tube dimensions and the number of drug coats adjusts total drug concentration [6e]

Resorbable Properties:

Protease XIV was used to assess in vitro enzymatic tube resorption and compared to PBS controls after incubation for 16 days (Figure 2). At day 3, there was a significant 250 % mass increase for both enzyme and control treated tubes due to media uptake (Figure 2A). Swelling and significant mass increase by day 3 leads to enzymatic access to silk cleavage sites and significant differences in mass remaining between protease and control treated tubes at day 13 (Figure 2A). At day 16, protease treated tubes degraded to 33 % mass remaining and control tubes with 100 %. In vitro enzymatic resorption does not directly correlate to *in vivo* resorption, but enables a standard for future projection of *vivo* resorption rates [7].

Drug-Elution Technology:

Drug-coated tubes possess antimicrobial properties; both killing (Figure 3A-C) and preventing growth (Figure 3D) of *Moraxella catarrhalis*, a bacterial strain commonly found in OEM [9]. Surrounding tube media was transparent for drug-coated tubes post- 24h bacterial culture, indicating efficacy with killing bacteria (Figure 3A-C). Antimicrobial properties were confirmed after subsequent culture of day 1 supernatant, where bacterial growth was prevented in drug-coated tubes (Figure 3D).

For time-dependent release data, HPLC methodology quantified drug eluted after incubation in PBS for 7 days (Figure 3, E-G). The total ciprofloxacin HCL and dexamethasone loaded were 80.3 and 272 μ g, respectively, and a faster release of 6h was observed for ciprofloxacin versus the sustained release of dexamethasone for 7 days (Figure 3, E-G). The hydrophobicity of dexamethasone was stabilized within the hydrophobic β -sheet domains of

the silk protein, enabling sustained release for 1 week, while the hydrophilicity of ciprofloxacin HCl resulted in burst release.

In vitro drug-elution profiles reveal efficacy and potential for localized drug release in vivo. The total drug concentration can be tuned in future designs with more drug coats. However, too many coats could pose stress on the TM and induce premature tube extrusion.

Proof-of-Concept in vivo study:

Chinchilla animal models were used to assess compatibility of silk ear tubes with clinical myringotomy procedures and in vivo resorption properties. Endoscopic images reveal tube patency in animal 1 and partial extrusion in animal 2 (Figure 4, A-B). The patent ear tube in animal 1 maintains structural integrity after 8 weeks and partial resorption (Figure 4A). Animal 2 presents a partially extruded ear tube with an unhealed myringotomy (Figure 4B). Tube dimensions or swelling are factors that could induce TM stress and lead to premature extrusion shown in animal 2 (Figure 4B).

Silk ear tubes reported here retained mechanical integrity for the entire 8 week implantation. Explanted tubes weights reveal about 48 % mass increase after 8 weeks (Figure 4C). Without histology, only speculation can be made regarding the cause of mass increase. Mass increase could be a result of cerumen uptake that was not removed during short-term collagenase digestion, but longer digestion risks digesting silk tube material. Preliminary in vivo assessment confirms feasibility of silk ear tube implantation and endoscopic images reveal promising long-term ear tube integrity (Figure 4, A-B).

Conclusions

The novel silk ear tube is the first proof-of-concept study with resorbable and drug-eluting properties. The preliminary in vitro and in vivo work show promise for tube efficacy and future clinical translation. This ear tube method has potential to eliminate extrusion-related complications and provides localized TM ear drop administration for expedited tissue healing. Future animal studies with long-term implantation and histological examination will provide stronger evidence for future clinical translation.

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References

- [1]. a) Rosenfeld RM, Schwartz SR, Pynnonen MA, Tunkel DE, Hussey HM, Fichera JS, Grimes AM, Hackell JM, Harrison MF, Haskell H, Haynes DS, Otolaryngol.--Head Neck Surg 2013, 149, S1b) Mai JP, Dumont M, Rossi C, Cleary K, Wiedermann J, Reilly BK, Laryngoscope 2017, 127, 956 [PubMed: 27796039] c) Djurhuus BD, Skytthe A, Christensen K, Faber CE, Int. J. Pediatr. Otorhinolaryngol 2014, 78, 1541. [PubMed: 25063508]
- [2]. Massey BL, Wen X, Rohr LR, Tresco PA, Dahlstrom L, Park AH, Otolaryngol.--Head Neck Surg 2004, 131, 921. [PubMed: 15577790]

- [3]. a)Park AH, Hoyt D, Britt D, Chase S, Tansavatdi K, Hunter L, McGill L, Sheng X, Skardal A, Prestwich GD, Laryngoscope 2013, 123, 1043 [PubMed: 23512811] b)Vlastarakos PV, Nikolopoulos TP, Korres S, Tavoulari E, Tzagaroulakis A, Ferekidis E, Eur. J. Pediatr2007, 166, 385 [PubMed: 17225951] c)Verhoeff M , van der Veen EL, Rovers MM, Sanders EA, Schilder AG, Int. J. Pediatr. Otorhinolaryngol 2006, 70, 1 [PubMed: 16198004] d)Hughes LA, Warder FR, F. R., Hudson WR, Arch. Otolaryngol 1974, 100, 151. [PubMed: 4843116]
- [4]. a)Dailey LA, Jekel N, Fink L, Gessler T, Schmehl T, Wittmar W, Seeger W, Toxicol. Appl. Pharmacol 2006, 215, 100 [PubMed: 16551473] b)Thurber AE, Omenetto FG, Kaplan DL, Biomaterials, 2015, 71, 145. [PubMed: 26322725]
- [5]. Rockwood DN, Preda RC, Yücel T, Wang X, Lovett ML, Kaplan DL DL, Nat. Protoc 2011, 6, 1. [PubMed: 21212779]
- [6]. a)Chen J, Zhan Y, Wang Y, Han D, Tao B, Luo Z, Ma S, Wang Q, Li X, Fan L and Li C, Acta Biomater 2018 80, 154. [PubMed: 30218777] b)Cheng G, Davoudi Z, Xing X, Yu X, Cheng X, Li Z, Deng H and Wang Q, ACS Biomater Sci Eng, 2018 4, 2704.c)Cheng G, Chen J, Wang Q, Yang X, Cheng Y, Li Z, Tu H, Deng H and Li Z, Nano Res, 2017 11, 3658.d)Lamboni L, Gauthier M, Yang G and Wang Q, Biotechnol Ad, 2015 33, 1855.e)Pritchard EM, Szybala C, Boison D and Kaplan DL,J Control Release 2010 144, 159. [PubMed: 20138938]
- [7]. Perrone GS, Leisk GG, Lo TJ, Moreau JE, Haas DS, Papenburg BJ, Lin SJ, Nat. Commun2014, 5, 3385. [PubMed: 24594992]
- [8]. a)Brown J, Lu CL, Coburn J, Kaplan DL, Acta Biomater2015, 11, 212 [PubMed: 25240984] b)Desai UH, Patwari AH, Maradiya JK, Sathawara MK, M. K., Suhagia BN, Rathod IS, Int. J. Pharm. Sci. Drug Res 2013, 5, 62.
- [9]. a)Gok U, Doymaz MZ, Bulut Y, Keles E, Yalcin S, Int. J. Pediatr. Otorhinolaryngol2001, 60, 49 [PubMed: 11434953] b)Faden H, Harabuchi Y, Hong JJ, J. Infect. Dis2018, 169, 1312c)Faden H, Duffy L, Wasielewski R, Wolf J, Krystofik D, Tung Y, J. Infect. Dis1997, 175, 1440. [PubMed: 9180184]

Figure 1. SEM images of machined control tubes (A) and drug-coated tubes (B-D). SEM of drug-coated tubes reveals smooth porous coatings painted on the exterior of machined tubes (B-D).

Figure 2.

Percent mass remaining of silk ear tubes as a function of incubation time in PBS (control) or 2 U/mL protease XIV. Measurement of weight at day 3 reveals ear tubes are still presenting excess weight due to water uptake. Two-way ANOVA with post-hoc Tukey test shows no significant difference for control tubes from day 0 to day 16, but significant difference between day 3 and 16 for protease treated tubes ($p < 0.05$). Holm-sidak t-test reveals significant difference in mass remaining between control and protease treated tubes at day 13 ($p < 0.05$). Representative images of control and treated tubes post-16 day incubation for control (B) and enzyme treated tubes (C) (N=3).

Figure 3.

Efficacy of tube drug elution. Ear tubes with (+D) and without drug (-D) were incubated 24 hours at 37 °C in LB and *Moraxella catarrhalis* (top row). Tubes with drug (C) reveal antimicrobial activity compared to the tube with no drug (B) and LB control (A). Subsequent culture of media for an additional 24 hours shows that tubes with drug are capable of both killing bacteria and preventing further growth (D, +D) (N=3). HPLC was used to quantify time-dependent release from the silk ear tube coated with ciprofloxacin HCL and dexamethasone. Total time to release 100% of the drugs reveals 6 h and 200 h for ciprofloxacin (E) and dexamethasone (F). Cumulative release of both drugs reveals sustained release of dexamethasone for the 1 week study, whereas ciprofloxacin HCL has a burst release within the first 6 h of incubation (G) (N=4).

Figure 4. Preliminary *in vivo* endoscopic examination at time of tube termination post-implantation for 8 weeks. Animal 1 reveals a patent tube and partially degraded ear tube, which corresponds to a partially healed myringotomy (A). Animal 2 presents a partially extruded tube and myringotomy site (B). Mass of silk ear tube explants at 8 weeks. Ear tubes were incubated in collagenase for 90 min to digest adhered tissue and placed in 37 °C for 24h to dry completely prior to measuring tube mass (N=4 total, N=2 for each time point).