A Novel Nanohydrogel Delivery System for Inner Ear Application

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INTRODUCTION

Hearing loss (HL) represents the most prevalent sensory disability worldwide, affecting over an estimated 275 million people. In the United States alone, it was estimated that at least 30 million adults suffered from some degree of HL in 2011. Currently, sensorineural HL (SNHL), which accounts for the most severe-to-profound HL cases, has no effective, non-invasive options available for treatment. The options are limited to sound amplification devices (hearing aids) and cochlear implants, which are limited by their variable effectiveness in patients or due to the need for invasive procedures, respectively.

Consequently, there has been an intense interest in the development of new approaches, including the use of molecular therapies. Delivering therapies to the inner ear, however, has always been a challenge for the Otolaryngologist mainly due to the limited cochlear blood supply and the poor penetration of drugs through the blood-inner ear barrier following systemic administration. Therefore, there is a need for the development of novel targeted delivery systems for the successful treatment of inner ear diseases.

In an effort to improve the efficiency of drug delivery across the round window membrane (RWM), our lab has developed a novel, stable, and controllable system that allows for delivery of a drug with improved outcomes. Specifically, we have successfully constructed a controlled and sustained local drug delivery system for inner ear disease including the delivery of gentamicin and dexamethasone, using a Chitosan-Glycerophosphate (CGP) thermosensitive hydrogel, which is biocompatible and biodegradable, and when applied directly to the round window niche (RWN), becomes a semi-solid gel that attaches to and persists in the niche. The results clearly demonstrated that the drugs could be slowly and steadily released from the CGP-hydrogel into perilymph through the RWM. More recently, chitosanase, was added as a regulator for this CGP delivery system in order to enzymatically “turn off” the delivery of therapy to the inner ear fluid vessels and vestibular structures when needed.1

Advances in biomedical nanotechnology, increased understanding of RWM diffusion properties, and discovery of novel therapeutic agents, have all sparked interest in the controlled local delivery of drugs and biomaterials to the inner ear using nanoparticles (NPs). The intratympanic (IT) approach is currently the most effective and promising route for non-invasive delivery of therapy to the inner ear as it allows for the diffusion of vasoactive agents, including drugs and NPs through the RWM.2,3

In the present study, we developed a novel CGP-based nanoparticle delivery system (nanohydrogel) for inner ear application and evaluated its structure and release kinetics in vitro. In addition, we evaluated if the nanohydrogel delivery system could be “turned off” using chitosanase. Finally, we evaluated the inner ear distribution of NPs following RWM application in a mouse model.

METHODOLOGY

Nanoparticles. Liposome nanoparticles were prepared using the thin-film hydration technique. Briefly, 10 mg of lipid (Avanti Lipids) were dissolved in chloroform (54% g-deoxycholate lip-L-Phosphatidylcholine, 40% cholesterol), 1.5% 1,1’-PFS100, PE, 1% 1,1’-Liss Rhodamine PE). The solution was vacuum-dried overnight, after which it was hydrated with 1X DPBS or a 0.2M solution of 50% ethanol/fluorescein (Sigma) for the self-quenching stability experiment. The hydrated lipids were then stirred at least 13 times using a mini-esterizer with a 0.1mM membrane (Avanti Lipids). NPs sizes were analyzed using a Zetasizer Nano range dynamics light scattering (DLS) machine (Malvern) and kept at 4°C in the dark until use.

CGP hydrogel. A 2% solution of high molecular weight chitosan (Sigma) in 0.1M HCl was prepared by magnetic stirring overnight at room temperature. Sample was kept at 4°C until use. To obtain a thermosensitive hydrogel for the downstream experiments, a 55% glycero-2-phosphate (Glycerol) was added droplets while stirring by hand until the pH of the solution reached 7.2 ± 0.1. The crosslinked chitosan gel obtained was a highly viscous thermosensitive CGP:hydrogel.

Nanohydrogel. The nanohydrogel was prepared by mixing CGP-hydrogel with the nanoparticles right after the crosslinking at a 1:10 ratio and hand stirring for at least 2 minutes. Samples were kept on ice or at 4°C for less than 1 hour, until use in in vitro or in vivo experiments.

Ultrastructure analysis. Samples of nanohydrogels were subjected to scanning electron microscopy (SEM) in an environmental Quanta 650 FEI Mark II scanning electron microscope at the Penn Regional Nanotechnology Facility (PRNF). In vitro stability and release kinetics of NPs. The stability and release kinetics were tested using various methods. Syringe models were constructed as previously described! The Rhodamine fluorescence of the collected PBS samples was measured using the TX-200 Molecular Imaging System (Biotek), and used as a surrogate for liposome release from the nanohydrogel. The diameter of the NPs before and after treatment was assessed using a Zetasizer Nano machine. Animals, C57Bl/6j mice (Charles River, Wilmington, MA) of either sex (<18 g) were used at 6 to 8 weeks of age. All procedures were conducted with approval from the IACUC of the University of Pennsylvania. Mice were anesthetized with a Ketamine/Xylazine (100/15) cocktail.

RESULTS

Structure of Nanoparticles

Figure 2. Structure of nanoparticles. The structure of nanoparticles consist of a phospholipid bilayer containing hydrophilic and hydrophobic components comprising the water solubilized in an aqueous core. The surface of the vesicles is functionalized with a specific fluorophore dye to serve as a marker for tracking the liposome release in vitro and in vivo.

Liposome stability

Figure 3. Release kinetics and integrity of liposomes. Sustained and controlled release of NPs was detected up to 7 days (top). Mean Fluorescence Intensity ± SEM is shown. The integrity was confirmed by measuring the NPs size in diameter (nm) before (stock) and after release using DLS (bottom and table). The results showed no significant size change.

Ultrastucture

Figure 5. Ultrastructural analysis. The ultrastructure of each component of the nanohydrogel delivery system was analyzed using environmental SEM. The liposome (left panel) were identified as spherical and relatively homogenous structures in a background of salt solution. The CGP:hydrogel (center panel), as expected, had the matrix like characteristic appearance, representing the cross-linked chitosan network. The nanohydrogel image (right panel) shows a hydrogel background with patchy surface representing the embedded liposomes.

SUMMARY

- We have successfully developed a nanohydrogel delivery system consisting of functionalized and stable liposomes embedded in a biocompatible, biodegradable, thermosensitive chitosan-hydrogel matrix.
- Our study suggests that this nanohydrogel system has the potential to safely deliver therapeutic agents in a controlled and sustained manner for inner ear application, and can be enzymatically regulated when needed.
- Importantly, this novel modular system could be further functionalized for targeted therapy for inner ear diseases that require safe and non-invasive delivery approaches.

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