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Intratympanic delivery of oligoarginine-conjugated nanoparticles as a gene (or drug) carrier to the inner ear

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ABSTRACT

A drug delivery system to the inner ear using nanoparticles consisting of oligoarginine peptide (Arg8) conjugated to poly(amino acid) (poly(2-hydroxyethyl L-aspartamide; PHEA) was investigated to determine whether the limitations of low drug transport levels across the round window membrane (RWM) and poor transport into inner ear target cells, including hair cells and spiral ganglion, could be overcome. Three types of carrier materials, PHEA-g-C18, PHEA-g-Arg8, and PHEA-g-C18-Arg8, were synthesized to examine the effects of oligoarginine and morphology of the synthesized carriers. Nile red (NR) was used as a fluorescent indicator as well as to model a hydrophobic drug. Compared with PHEA-g-C18-NR nanoparticles, the oligoarginine-conjugated nanoparticles of PHEA-g-C18-Arg8-NR and PHEA-g-Arg8-NR entered into HEI-OC1 cells at significant levels. Furthermore, the strongest fluorescence intensity was observed in nuclei when PHEA-g-C18-Arg8 nanoparticles were used. The high uptake rates of PHEA-g-C18 and PHEA-g-C18-Arg8 nanoparticles were observed in *ex vivo* experiments using hair cells. After the delivery of PHEA-g-C18-Arg8 nanoparticles with reporter gene transfer, EGFP (enhanced green fluorescent protein) expression was monitored as an indicator of gene delivery. In the inner ear cells, PHEA-g-C18-Arg8 nanoparticles showed comparable or better transfection capabilities than the commercially available Lipofectamine reagent. PHEA-g-C18-Arg8 penetrated *in vivo* across the RWM of C57/BL6 mice with Nile red staining and GFP expression in various inner ear tissues. In conclusion, PHEA-g-C18-Arg8 nanoparticles were successfully transported into the inner ear through the intratympanic route and are proposed as promising candidates as delivery carriers to address inner ear diseases.

1. Introduction

The intratympanic delivery of nanocarriers would provide an opportunity to treat sensorineural hearing loss or at least prevent serious progression of hearing loss originating from damage to hair cells and spiral ganglion neurons (SGN) in the cochlea of the inner ear [1]. Many drugs have been developed in attempts to induce regeneration of hair cells and thus restore hearing [2]. Unfortunately, however, few drugs can reach the target site of action at therapeutic concentrations in the inner ear [3] because of efflux pump proteins (e.g., p-glycoprotein) expressed in the blood–labyrinth barrier (BLB) [4,5]. Further, therapeutic levels of drugs in the inner ear may require high systemic doses, which are often associated with undesirable side effects. Thus, strategies for intracochlear delivery will attempt to substantially increase the drug bioavailability in the inner ear and would have the highest efficiency among inner ear delivery methods, including perforation and surgical manipulation of the ear, which have significant risk of deafness [6–8].

Therefore, the intratympanic route has attracted a great deal of interest for local drug delivery, performed via the injection or perfusion of drugs into the middle ear and drug diffusion into the inner ear through the round window membrane (RWM) [9]. This local delivery approach has several advantages, including high drug concentrations in inner ear fluids and the avoidance of undesirable

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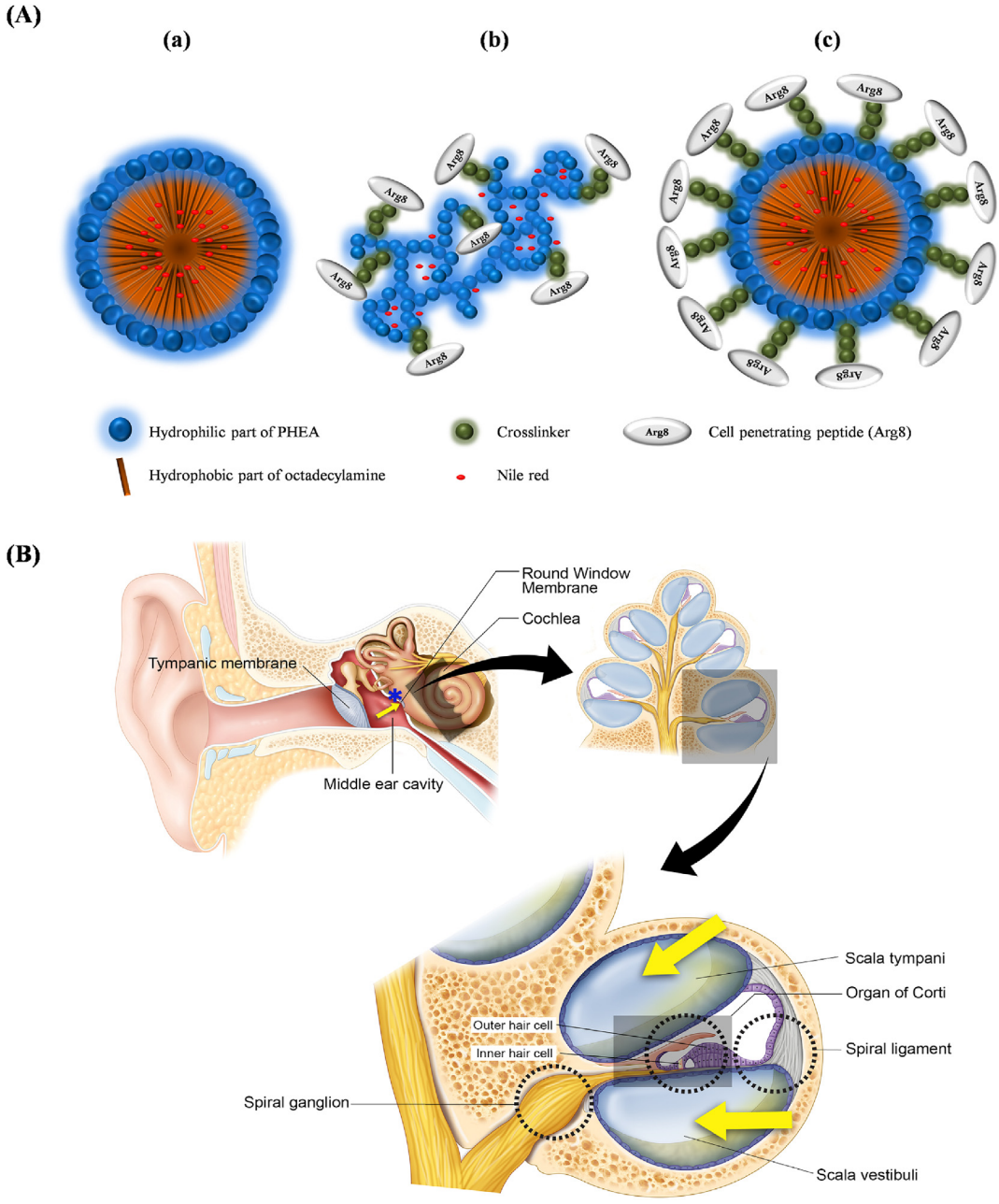
systemic exposure [10–12]. Obstacles to intratympanic drug delivery include anatomical barriers, such as drug absorption from the middle ear to the inner ear, including the RWM due to narrow tight junctions of the outer epithelial layer, and drug loss in the middle ear through the Eustachian tube [9,13]. Therefore, effective drug delivery to the inner ear would be dependent on the permeability across the RWM and the contact time of the drug solution with the RWM without loss through the Eustachian tube [9].

Nanoparticles conjugated with cell penetrating peptide (CPP), a short sequence < 30 amino acid residues in length [14], have the ability to cross membranes, such as the RWM [15]. Nuclear delivery induced by cationic nanoparticles, such as oligoarginine peptide (Arg8) nanoparticles, may be an attractive option to improve transgene expression and delivery of genes, including DNA or siRNA, into the nuclei of target cells [16–18].

In this study, a graft copolymer delivery system was designed to investigate the possibility of use as a drug and gene delivery system into the inner ear, as shown in Scheme 1(B), and the potential nuclear entry of poly(2-hydroxyethyl L-aspartamide; PHEA)-based nanoparticles in cochlear cells. Nanoparticles formed by self-assembly in aqueous solution of a novel biodegradable amphiphilic graft copolymer, PHEA, as the backbone polymer conjugated with Arg8 as a CPP. We prepared three types of graft copolymer: (1) PHEA with octadecylamine (C18) (PHEA-g-C18) as a control, (2) PHEA-g-Arg8, (3) PHEA-g-C18-Arg8. These graft copolymers were used in a series of *in vitro*, *ex vivo*, and *in vivo* experiments as drug and gene delivery materials into inner ear target sites.



Scheme 1. (A) Schematic structures of Nile red-loaded (a). PHEA-g-C18, (b). PHEA-g-Arg8, and (c). PHEA-g-C18-Arg8 particles (B) The location of target cells (spiral ganglion, organ of Corti, spiral ligament) and delivery route of PHEA-based nanoparticles in the inner ear through the round window membrane.