Imidazole-modified chitosan nanoparticles for delivery to lung epithelial cells in air-liquid interface cultures

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Statement of Purpose: Drug delivery to the lungs has many advantages over more conventional delivery methods. For lung diseases such as asthma, cystic fibrosis, and chronic obstructive pulmonary disorder (COPD), pulmonary delivery of pharmaceutical agents represents a site-specific method of distribution that creates high local concentration, while also maintaining low systemic exposure and avoiding first-pass metabolism[1]. Unfortunately, it has been shown that current delivery efficiencies for dry power inhalers range from 3 to 30% for children and adults, which is, in part, due to the inability of drug particles to traverse the mucosal layer. The specific properties of the particles used in these inhalers, including size and charge, determine their effectiveness when used in pulmonary delivery, and thus careful optimization of these characteristics would allow us to increase delivery efficiency[2]. Chitosan is a biopolymer derived from chitin, whose relatively low toxicity and biocompatible profile make it an ideal drug carrier for a range of applications[3]. Our group has previously synthesized an imidazole-modified chitosan (chitosan-IAA) which enhances biocompatibility and mucosal penetration[4], but has not been characterized for use in airways. Previous studies of chitosan have only characterized it as a particle in liquid culture, which lacks important mucosal characteristics that are present when cells are grown at the air liquid interface. In this study we seek to investigate how imidazole modification can effect transport of nanoparticles across airway mucosal surfaces.

Methods: Imidazole-modified chitosan was created through the addition of imidazole acetic-acid (IAA) to the primary amines of 83% de-acetylated chitosan (Novamatrix) through EDC/Sulfo-NHS chemistry. Nanoparticles were formed from the resulting chitosan-IAA via electrostatic interactions with tri-polyphosphate. Size and zeta potential characterization were done on a Malvern Nano ZS Zetasizer. Particles were tagged with VivoTag 645 (Perkin Elmer), and particle uptake was investigated by treating A549 cells with concentrations ranging from 6 μg/ml to 40 μg/ml. Quantification was completed through flow cytometry and microscopy after 24 hours. Air-liquid interface cultures (ALI) were created by growing A549 cells on collagen-coated transwells (Corning). Cells were grown to confluence in liquid culture media (Lonza) and then air-lifted and grown for 3-4 weeks in differentiation medium (Lonza). Cells were stained for Mucin 5AC, Lung surfactant A, beta-tubulin IV, and ZO1 tight junctions. These cultures were treated with tagged particles at 20 μg/ml.

Results: We created unmodified chitosan particles, as well as chitosan-IAA particles modified at 8% and 18%. The size of all of these particles was 60-70 nm. The zeta potential of all three particles is approximately +30 mV. We treated A549 cells with these tagged particles and saw near 8-fold increases in particle uptake at low concentrations in comparison to conventional chitosan particles, data is shown in figure 1A/B. Cells grown at air liquid interface were also treated with these particles at a concentration of 20 μg/ml. We saw that 18% modification caused a significant decrease in particle uptake over time in comparison to both convention chitosan particles and those with 8% modification. We did not see significant differences at 5 hours(Figure 1C). Figure 1D shows staining of a transwell culture for proteins characteristic of a mucosal layer (mucin) and ciliated cells (beta-tubulin IV).

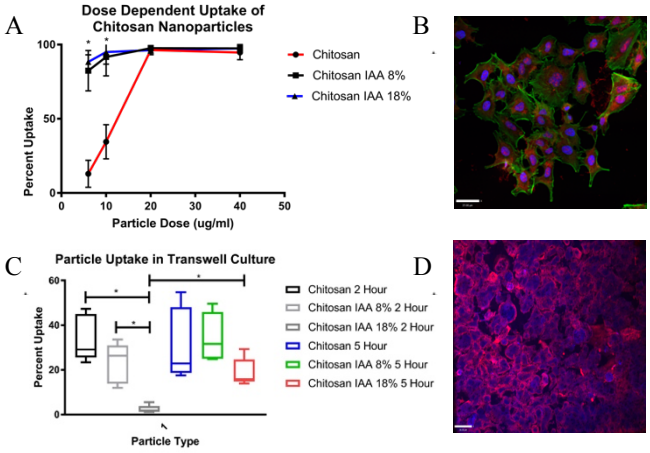

Figure 1: (A,B) Uptake of modified and un-modified chitosan particles in liquid culture, (B) green-actin, red-particles, blue-DAPI. (C) Uptake in transwell culture. (D) Staining of transwell culture, blue-Mucin 5AC, red-beta-tubulin IV. (*$p<0.05$, n=6 for both, One-Way ANOVA, Tukey's multiple comparison's test)

Conclusions: These results show that our modified chitosan-IAA particles are being taken up by A549 cells at low concentrations more than conventional chitosan particles. This could mean that we could achieve similar therapeutic outcomes with lower doses of nanoparticles, avoiding any cytotoxicity that might be associated with higher doses, though further studies will have to be done to see if this correlation exists. In air-liquid interface culture, which more closely mimics in-vivo conditions, we saw a decreased uptake rate of the 18% modified particles in comparison to the 8% and unmodified particles; this could be due to trouble crossing the mucosal surface itself, or simply slower uptake by the underlying epithelial cells. Future studies will focus on separating out this difference to determine how these particles can be used therapeutically. We will also be looking at the ability of these particles to silence genes in air-liquid interface cultures, which will later lead to the creation of a transwell lung cancer model for treatment using siRNA.

References: [1] Beck-Broichsitter, M. et al., J. Control. Release. 2012;161:214–224.[2] Garcia, A. et al. J. Drug Deliv. 2012; 2012:1–10. [3] Mao, H.-Q. et al. J. Control. Release. 2001;70:399–421 [4] Ghosn, B. et al. Oligonucleotides. 2010 Jun;20(3):163-72