Inhalable Dry Powder Microparticles for Delivery of Bacteriophages to Treat Cystic Fibrosis Associated Bacterial Lung Infections

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Statement of Purpose: Lung airway diseases are a major cause of infant death and are associated with clogging of airways due to mucus build-up, decreased mucociliary clearance, chronic inflammation and bacterial infection[1]. Polymeric particles have emerged as an ideal platform for pulmonary delivery of various hydrophobic and hydrophilic drugs to be encapsulated and released over long periods of time. This research focuses on engineering novel particles to deliver bioactive bacteriophages to the infected cystic fibrosis (CF) lung. Phages do not infect mammalian cells and have been used safely in humans for several decades.

Methods: Porous PLGA particles were prepared by double emulsion of water in oil in water (w/o/w). Density of particles was calculated using a Coulter counter. Bacteriophages were grown, purified and quantified as previously described [3]. Bacteriophages were adsorbed on the particle surface by incubating with particles for 3 hours on a shaker and washed extensively. Dry powder formulations were obtained by lyophilization in presence of lactose as a cryoprotectant. For lung insufflation, fluorescently labeled PLGA particles were mixed with inhalation grade lactose (ML006, DFE Pharma) and were then delivered to mice lungs through intra-tracheal route using a Penn-Century insufflation device. Each mouse received a dose of 0.5 mg of PLGA particles and was then imaged using IVIS or FMT. To retrieve phages from lungs, lungs were explanted, homogenized and the lysate was tested using plaque assay.

Results: Porous PLGA particles were successfully prepared (Fig. 1). The resulting particles were highly porous. Density of particles was 0.25-0.30 g/cc while their mean diameter was in the range of 8-12 µm. Thus their aerodynamic diameter was around 4-6 µm making them ideal for deep lung deposition and avoiding clearance by alveolar macrophages[2]. Porous microparticles provide high surface area for phages to adsorb to. Phage titers with particles loaded with 2 different types of phages were in the order of 10^5 phages/mg of particles. Fig. 1C shows the zoomed in images of co-localization of particles carrying phages in plaques, which are regions where bacterial colonies have been destroyed by bacteriophages. This assay demonstrated that phages loaded into particles retained their bactericidal activity. We further investigated the aerosolization properties of our particles in mice. As shown in Fig. 2A, particles accumulated throughout the lungs of the mice showing aerosolization and deep lung deposition. We then tested the activity of delivered phages in explanted lungs. As shown in Fig. 2B, phages loaded in particles were successfully delivered in lungs and were found to be active. Particle tracking showed that PLGA particles are cleared within 20 hours from healthy mice lungs (Fig. 1F-G).

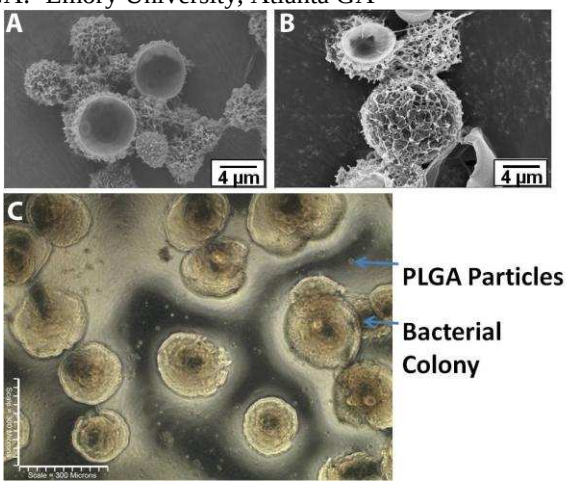

Figure 1. A-B): SEM micrographs of porous PLGA particles. C) Zoomed in image of plaques showing co-localization of PLGA particles with plaques.

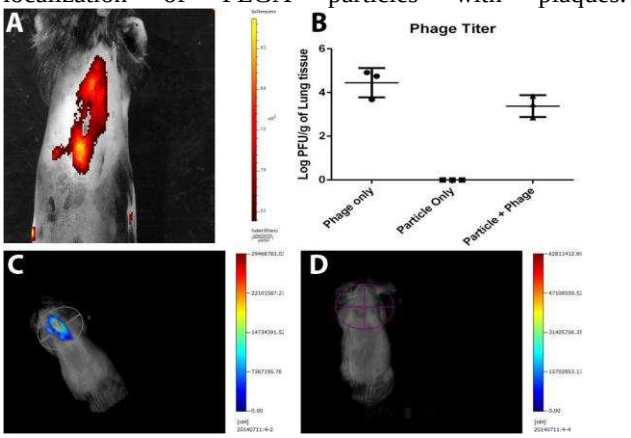

Figure 2. A) IVIS image showing delivery of fluorescent particles to mice lungs. B) Graph showing amount of active phages in explanted lungs. C-D) FMT images showing clearance of particles from lungs in 20 hours.

Conclusions: We have shown our ability to fabricate porous PLGA microparticles, capable of depositing in lungs after dry powder insufflations. Bacteriophages can be loaded on microparticles and when delivered on microparticles were found to be active in mice lungs. Such inhalable particle-bacteriophage system can allow treatment of persistent and life threatening lung infections in cystic fibrosis and other airway diseases. Other therapeutic drugs can also be encapsulated and delivered via these particles.

References:

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