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Aerosol droplet delivery of mesoporous silica nanoparticles: A strategy for respiratory-based therapeutics

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Abstract

A highly versatile nanoplatform that couples mesoporous silica nanoparticles (MSNs) with an aerosol technology to achieve direct nanoscale delivery to the respiratory tract is described. This novel method can deposit MSN nanoparticles throughout the entire respiratory tract, including nasal, tracheobronchial and pulmonary regions using a water-based aerosol. This delivery method was successfully tested in mice by inhalation. The MSN nanoparticles used have the potential for carrying and delivering therapeutic agents to highly specific target sites of the respiratory tract. The approach provides a critical foundation for developing therapeutic treatment protocols for a wide range of diseases where aerosol delivery to the respiratory system would be desirable.

Key words: Mesoporous silica nanoparticles; Aerosol droplets; Respiratory tract

Introduction

Multifunctional engineered silica nanocarriers can effectively transport a wide range of specific therapeutic agents to control delivery, timing, and precision of compounds to biological target sites.[1-7] However, their use via inhalation is currently limited by a general lack of technological development to deliver aerosolized nanoparticles that are inhalable and controllable for optimal delivery to selective sites throughout the respiratory tract. The need for efficiently designed nanocarrier systems is

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crucial to appropriately target a therapeutic compound and protect it so it could be released upon reaching the desired site.[8-11] This is especially true in the lung due to a complex airway geometry, variations in breathing patterns, specific cells at target sites and factors that affect particle deposition, including size, shape, charge and density.

Mesoporous silica nanoparticles (MSNs) are inorganic-based nanocarriers developed for hydrophobic and hydrophilic drug molecules, as well as other therapeutic elements for controlled on-demand delivery in biological systems. MSN drug-carrier technology has advanced to the pre-clinical phase and shows significant potential for treating diseases by limiting side effects and controlling drug release.[12-15] To date, MSN delivery applications primarily use intravenous injection (IV). While IV is a well-established therapy for nanocarrier drug delivery, inhalation represents a highly desirable route of delivery to specifically target the respiratory system. Respiratory diseases also currently rank among the top ten causes of death globally.[16] Current research in MSN therapeutics has demonstrated that inhalation is a possible route of delivery, specifically for lung cancer,[17] as well as for novel applications for the treatment of tuberculosis.[13] Efficient and sustained delivery of therapeutic compounds carried and retained in the lungs for controlled release also represents a new approach for treatment.

The purpose of this study was to generate a functional aerosol containing unaggregated forms of MSNs with the potential to be equipped with a broad-range of disease-targeting components.[15] The specific goals of the study were creation of suitable aerosolization conditions, verification of limited-to-no toxicity, while demonstrating MSN integrity to widely deliver nano bio-functional components to highly diverse regions of the respiratory tract. To optimize drug delivery and deposition in all areas of the respiratory tract, MSN was suspended in nanopure water and aerosolized in droplets in the respirable size range (0.1 to 3.0 μm). A mouse-model was used to test the inhalability of MSN. The effectiveness of the design was evaluated on the basis of deposition in pulmonary tissues, as well as cells collected from the entire respiratory tract and imaged with fluorescent and electron microscopy. Toxicity at each level of the respiratory tract was evaluated to assess acute toxicity of the combined nano-aerosol delivery biotechnology. To our knowledge, this represents the first comprehensive safety profile of aerosolized MSN in an *in vivo* inhalation model.

Methods

MSN synthesis

The synthesis of 50 nm mesoporous silica nanoparticles was based on a previously published method.[15] Briefly, 250 mg cetyltrimethylammonium bromide (CTAB) and 220 mg Pluronic F127 were mixed with 120 mL of H_2O, to which 875 μL of 2 M NaOH aqueous solution was added. The solution was kept at 80 °C before 1.2 mL of tetraethyl orthosilicate (TEOS). This was followed by an addition of 300 μL of trihydroxysiylpropyl methylphosphonate after 30 min. The resulting suspension was then stirred for 2 h and the particles were collected by centrifugation. The particles were then resuspended in a solution of 60 mL methanol with 60 mL of H_2O and mixed with 0.8 g of NH_4NO_3. After stirring for 30 min at 60 °C, the particles were centrifuged and washed with methanol.

Polymer coating and fluorescent labeling

To perform polymer coating, 100 mg of particles were suspended in 10 mL of 2.5 mg/mL polyethyleneimine (PEI) ethanolic solution and the solution was stirred at room temperature for 30 min. The particles were collected by centrifugation and washed with ethanol. 20 mL of anhydrous dimethylformamide (DMF) was used to resuspend the PEI-treated particles, and 1 mg of fluorescein isothiocyanate (FITC) N-hydroxysuccinimide (NHS) ester was added into the solution. 12 h later, 500 mg of activated m-polyethylene glycol (PEG) was added and the solution was stirred for another 12 h. The resultant particles were centrifuged and washed with DMF, methanol and water. The final suspension of MSN for aerosolization and inhalation studies was in nanopure water.

Physiochemical characterization

Images were taken using a JOEL 1200 transmission electron microscope. Nanoparticles were suspended into a 50 μg/mL methanol suspension. Approximately 20 μL of the solution was then used for sample preparation. Dynamic light scattering was performed on a ZetaSizer Nano (Malvern Instruments Ltd., Worcestershire, UK) using a 40 μg/mL aqueous suspension to determine the particle size.

MSN aerosol generation

A nanopure water droplet aerosol containing MSN nano-particles was delivered simultaneously to individual mice during 5 h using a version of a multi-port exposure apparatus.[18] This aerosol was generated using a MiniHeart nebulizer[19] (Westmed, Inc., Tuscon, AZ) operated at 39 psig with filtered compressed air. The nebulizer was placed in an ice-water bath at 0 °C to minimize evaporation. The output concentration of liquid aerosol was about 106 μL/min (with only 1 or 2 μL/min of water vapor with the nebulizer in an ice-water bath). The optimal concentration of the MSN nano-particles in the nebulizer to minimize foaming of the aerosol was found to be 4 mg/mL (4 μg/μL), and the nebulizer output of MSN was 424 μg/min in 2 L/min of air. Since there was no diluting air, the aerosol MSN concentration was 424 μg/min divided by 2 L/min of air at 212 μg/L. When entering the exposure chamber at ambient temperature of about 25 °C, the water droplet aerosol had a mass median aerodynamic diameter of about 1.8 μm.

The mass of aerosolized particles deposited in the lungs of a mouse was estimated by multiplying the amount inhaled by the deposition fraction for the selected region of the respiratory tract.[20]

The droplet deposition in the mouse respiratory tract in this study can be calculated by: Dose deposited = fctv, where:

- f fraction deposited in respiratory tract region (function of particle size) given for the mouse pulmonary region as 0.08 for 1.8 μm diameter water droplets.[20]
- c aerosol MSN concentration (micrograms per liter of air): 212 μg/L.