Assessing The Effect Of Inhaled Particle Size On Mucociliary Transit Activity In Live Mice

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Background

- The CFTR ion channel defect in airway epithelial cells reduces airway surface liquid (ASL) depth and impairs mucociliary transport (MCT).
- We have created non-invasive, synchrotron imaging-based methods for quantifying ASL and MCT as direct measures of airway health.
- Our *in vivo* MCT quantification method relies on tracking the motion of deposited high refractive index (HRI) glass marker particles, which have uniform size, shape and surface properties.
- Previous studies have suggested that MCT rate is dependent on particle material, but the effects of particle size were unclear.
- The aim of this study was to determine whether particle size affects MCT rate and behaviour.

Methods

- Intubated and ventilated C57Bl/6 mice (n=14) were imaged on the BL20XU beamline at SPring-8 Synchrotron in Japan (See Fig 1).
- A mix of two differently-sized HRI marker particles (10 µm and 33 µm dia, Corpuscular, USA) were delivered to the lower trachea using a Dry Powder Insufflator™ (PennCentury, PA).
- Images of the trachea with an effective pixel size of 0.56 µm and a field of view of 1.43 mm x 1.2 mm were captured using a scintillator and high-resolution camera.
- After baseline imaging (room-air respiration) an Aeroneb nebuliser was used to deliver aerosol (n=8 hypertonic & n=6 isotonic saline) for 15 msec on each inspiration (120 br/min) for 15 min.
- Images were collected at 5 Hz in 30 second blocks, every minute.
- Custom semi-automated image tracking software was used to tag particles, and calculate individual and bulk transit rates.

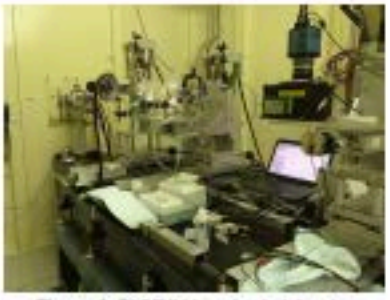

Figure 1: BL20XU imaging hutch setup

Results

- Most HRI particles remained stationary throughout the imaging period. This effect was much more pronounced than with other particles that we have examined previously (quarry dust, fibreglass, asbestos, lead,etc).
- Moving particles were only detected in 6 mice treated with hypertonic saline, and 2 treated with isotonic saline.
- Most bead movement was localised to the dorsal tracheal surface.
- When data was pooled across all time-points, there was a statistically significant difference in mean MCT rate between the large and small particle groups (1.36 and 1.89 mm/min, respectively, $p<0.05$, t-test). Insufficient moving particles were detected at each individual time-point for statistical analysis.

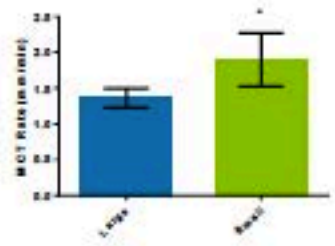

Figure 2: Effect of HRI bead size on MCT rate

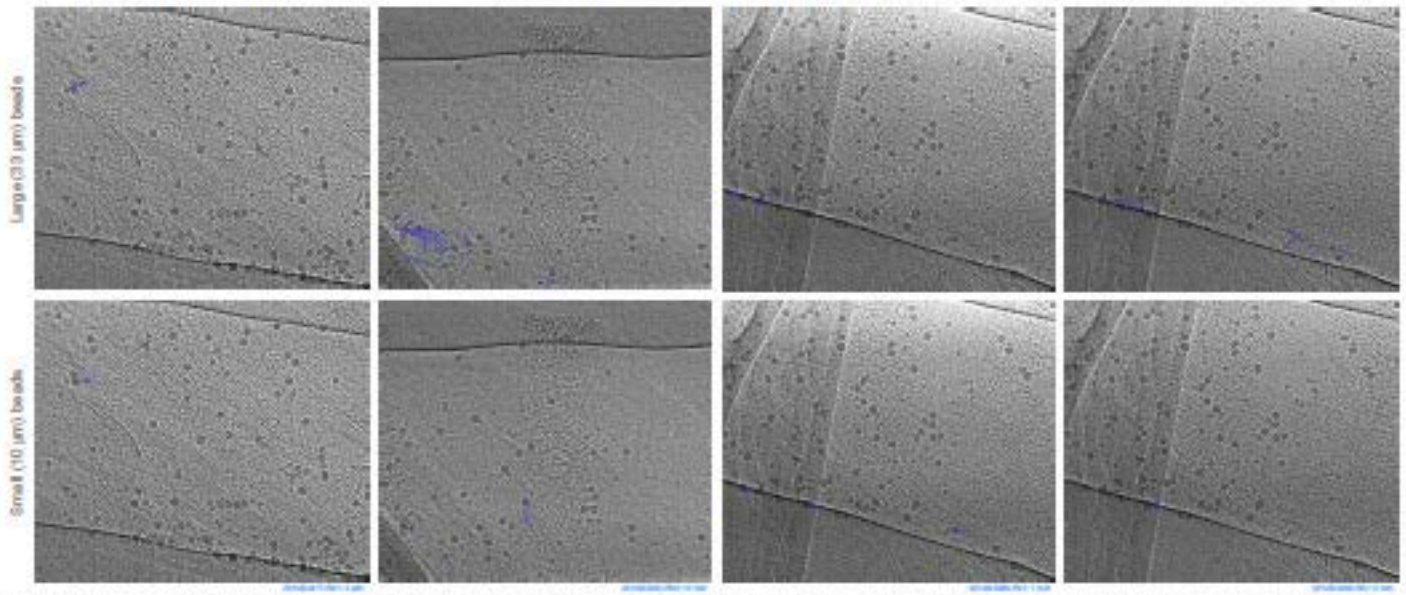

Figure 3: High magnification images of high refractive index glass bead MCT marker particles in the trachea of live mice. The top row of images show the marked trajectories of small (10 µm) beads, and the bottom the large beads (33 µm) at the same time-point in the same animals. The head is to the right and the spine is toward the bottom. Imaging location is just above the carina. Each moving particle is identified with a red mark, with its location in subsequent frames identified with blue marks. Stationary particles are not marked. Images 1.43 x 1.2 mm.

Conclusion

- MCT rate in live mouse trachea for these glass beads appears to be dependent on particle size, but surprisingly few of the introduced beads moved during the analysis period.
- We speculate that sub-optimal airway surface hydration and/or HRI bead surface properties may have influenced the ability of most particles to move during the imaging period.
- Future studies (Nov 2015) will examine the effect of changing inhaled air humidity and particle surface coatings on particle MCT.

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