NON-INVASIVE AIRWAY HEALTH MEASUREMENT USING SYNCHROTRON X-RAY MICROSCOPY OF HIGH REFRACTIVE INDEX GLASS MICROBEADS

Martin Donnelley[1-4], Kaye Morgan[5], Karen Siu[5,8], Nigel Farrow[1-4] and David Parsons[1-4]

1. Alan Scott CF Research Laboratory, Respiratory and Sleep Medicine, Women's and Children's Hospital, South Australia
2. Robinson Research Institute, 3. School of Paediatrics and Reproductive Health, and 4. Centre for Stem Cell Research, University of Adelaide, South Australia
5. School of Physics, Monash University, Victoria
6. Australian Synchrotron, Victoria

BACKGROUND:

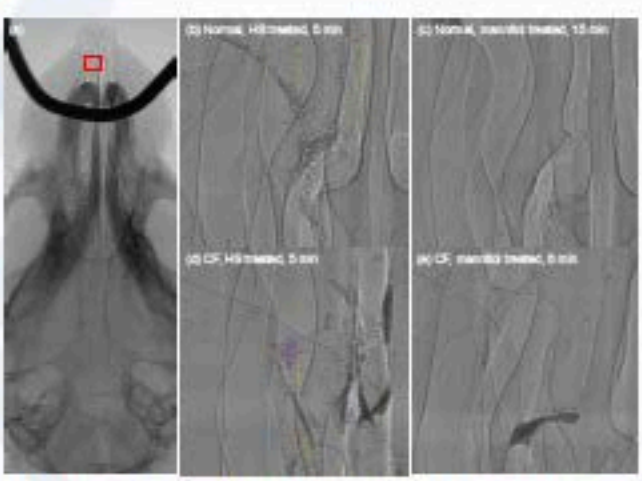
- Cystic fibrosis (CF) is caused by a defective CF transmembrane conductance regulator gene that results in defective ion-transport across the airway epithelium, compromising the ability of the mucociliary transit (MCT) system to clear the airways of debris and pathogens. Lung disease starts early in childhood and relentlessly progresses, producing dramatic reductions in quality of life, as well as an early death from lung failure.
- We have developed a synchrotron X-ray microscopy method that rapidly, directly, and non-invasively measures the rate and patterns of MCT behaviour to directly characterise airway health and the effects of treatments [1].
- Although the nasal airways of CF mice exhibit the CF pathophysiology, there is evidence that nasal MCT is not altered in CF mice [2].
- The aim of this experiment was to determine if our non-invasive airway health assessment method could identify any differences in nasal MCT rate between normal and CF mice potentially lost in bulk MCT measurements.

METHODS:

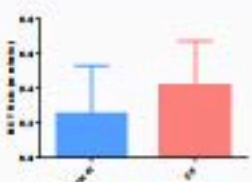
- Experiments were performed on the BL20XU beamline at the SPring-8 Synchrotron in Japan.
  - Monochromatic 25 keV X-rays
  - Propagation distance: 1 metre
  - SPring-8 BM3 (x10) and pco.edge sCMOS detector
  - Field of view: 1.43 mm x 1.2 mm with isotropic pixel size: 0.56 µm
  - Exposure length: 10 ms
- Mice (n=20 normal, n=21 CF) were anaesthetised and a small quantity of 22 µm high refractive index (HRI) glass beads (Corpuscular, USA) were insufflated into the nasal airways using a Dry Powder Insufflator™ (PennCentury, USA).
- Mice were placed onto the hutch x-y-rotation stage for imaging. Images were acquired at 5 Hz in 15 sec blocks every minute for 15 minutes.
- After baseline imaging, the nasal airways were treated with a common clinical CF airway rehydrating therapy:
  - Aerosolised hypertonic saline (HS), or
  - Dry powder mannitol
- Custom software was used to manually locate and track moving particles (observer blinded to group & timepoint), and calculate MCT rates.

RESULTS & DISCUSSION:

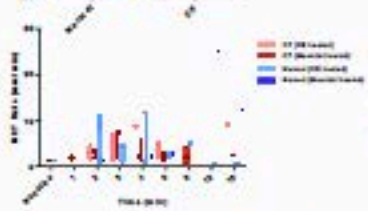
- Seven mice (n=2 normal, n=5 CF) were excluded due to the presence of too many particles to enable accurate HRI bead tracking.



(F1) (a) Image of the mouse skull showing the region of interest marked with a red box. (b-e) Example high magnification images of HRI glass beads in the nose of four live mice. Moving particles are identified with a red cross, with locations in subsequent frames marked with blue circles. Stationary particles are not marked. The example in (c) shows a rapidly moving outlier bead. Images are ~ 1 mm x 1 mm.

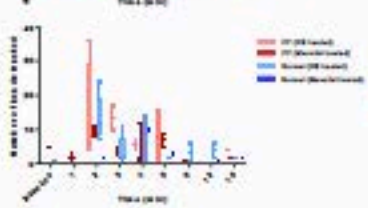


(F2) Baseline MCT rate (treatment groups pooled) showed no statistically significant differences in MCT rate between untreated CF and normal mice.



(F3) MCT rate of all moving particles over time shows that MCT activity begins to increase ~2 min after treatment delivery. Additional statistical analyses are currently being performed.

□=25%-75%, I=Min-Max



(F4) Total number of moving particles tracked at each time-point. The greatest number of moving particles were detected 2 min after treatment delivery.

- No differences in nasal MCT between CF and normal mice were detected.
- Relatively few particles moved compared to the number delivered, and we hypothesise this location in the nose may capture beads.
- The anatomical complexity of the nasal airway complicates tracking.

CONCLUSION:

- Using this new imaging tool we plan to assess:
  - MCT in other locations (posterior nose and trachea).
  - The efficacy of other clinically relevant therapies for CF.
  - Alternative models of CF–like disease (e.g. β-ENaC lungs).
- The improved sensitivity provided by this technique will accelerate the ability to identify useful CF lung disease-modifying interventions in small animal models, and enhance the development and efficacy of proposed new therapies.

REFERENCES:

[1] M. Donnelley, et al., "Non-invasive airway health assessment: Synchrotron imaging reveals effects of rehydrating treatments on mucociliary transit in-vivo," *Scientific Reports*, vol. 4, Jan 14 2014.

[2] B. R. Grubb, et al., "Mucociliary transport determined by in vivo microdialysis in the airways of normal and CF mice," *American Journal of Physiology - Lung Cellular & Molecular Physiology*, vol. 286, pp. L588-95, Mar 2004.

ACKNOWLEDGEMENTS:

SPring-8 proposal: 2013B1734.

Experiment Funding: Pharmaxis, NHMRC, WCH Foundation and Cure4CF Foundation.

Experiment Travel: Australian Synchrotron ISAP.

Conference Travel: Robinson Institute.