ADVANCES IN AIRWAY SURFACE IMAGING FOR CYSTIC FIBROSIS: EXTENDED MONITORING OF INDIVIDUAL PARTICLE MUCOCILARY CLEARANCE

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BACKGROUND:

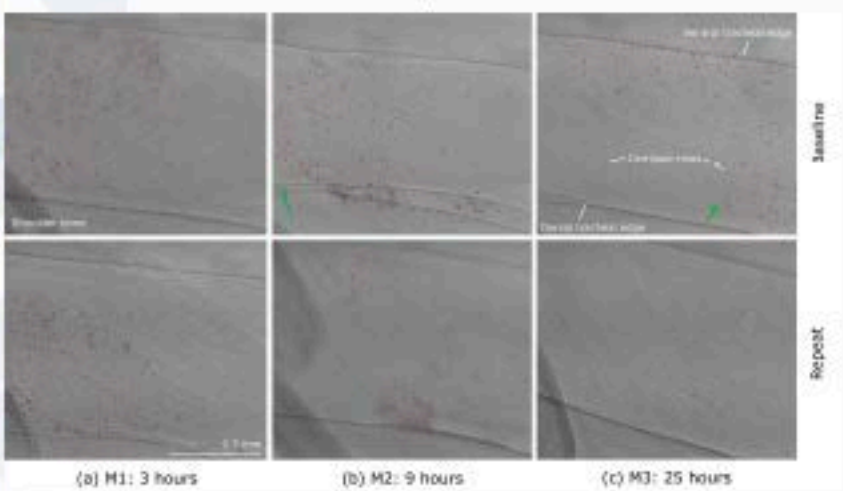
- The CFTR ion channel defect in epithelial cells lining the airways impairs mucociliary transport (MCT).
- Quantification of MCT is traditionally performed using radio-labelling or dye transit techniques, but these only provide bulk measures and are relatively insensitive and unsuited to topographically-complex airways.
- **We can now directly measure MCT in vivo using deposited marker particles and high-magnification synchrotron phase contrast X-ray imaging with a magnification / resolution at least two orders of magnitude higher than other current methods such as μ-CT and MRI.**
- Particle clearance by MCT is a slow process occurring over hours to days, however our previous studies have only examined MCT over ~20 minutes after dosing. Reductions in radiation dose now allow the use of repeated-measures study designs to follow changes in individual animals.
- **This study examined the MCT of deposited marker particles in the lungs of live mice for up to 25 hours.**

METHODS:

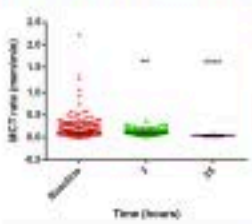
- Intubated (flexiVent ventilation) C57Bl/6 mice were imaged on the BL20XU beamline at SPring-8 (Japan).
- Lead marker particles were delivered to the trachea using a Dry Powder Insufflator™ (PennCentury, PA).
- Images of the trachea with an effective pixel size of 0.56 μm and a field of view of 1.43 mm x 1.2 mm were captured using a high-resolution camera. All images used a 50 ms exposure:
  - Baseline: (12 images 5 sec apart) x 2 runs 5 min apart.
  - Repeat: 110 images 5 sec apart.
- Baseline imaging determined the initial particle distribution and behaviour. Mice were then allowed to recover from anaesthesia.
- After 3, 9 or 25 hours (n = 8 per group) the mice were re-anaesthetised and imaged again to determine if there was any alteration in the number of particles present, and whether the nature of the particle MCT behaviour had changed.
- Custom software was used to locate particles, and calculate MCT rates.

RESULTS:

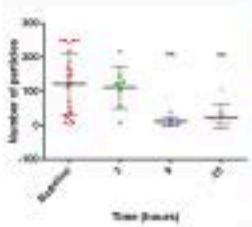
- The repeat imaging protocol was well tolerated, with no discernable effects from the radiation exposure.
- Both qualitative and quantitative visual analyses show that substantially fewer particles are present at the later time-points, and that the MCT rate was reduced at the later time-points.



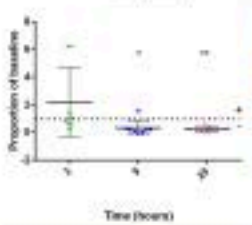
(F1) High magnification images of lead dust in the trachea of three live mice (M1-M3) after particle insufflation, assessed using a repeat imaging study design. The top row of images are at baseline, shortly after lead dust was delivered to the airway surface. The bottom row shows the same location (a) 3 hours, (b) 9 hours and (c) 26 hours later. The head is to the right and the spine is toward the bottom. Imaging included the same bone edge (bottom LH corner) to ensure the same airway region was examined each time. Imaging location is just above the carina. Stationary particles are marked in red and (the few) moving particles in green (arrows).



(F2) MCT rate of all moving particles over time. Note that no moving particles were detected at 9 hours. The MCT rate at 9 and 26 hours was significantly reduced compared to baseline
***p<0.01, ****p<0.0001, Kruskal-Wallis One way ANOVA with Dunn's multiple comparisons.*



(F3) Total number of particles (either moving or stationary) at each time-point. The total number of particles present at 9 and 26 hours was significantly reduced compared to baseline.
***p<0.01, Kruskal-Wallis One way ANOVA with Dunn's multiple comparisons.*



(F4) Proportion of particles (moving and stationary) at each repeat imaging point, compared to baseline. Significantly less particles remained at 26 hours compared to 3 hours.
**p<0.05, **p<0.01, Kruskal-Wallis One way ANOVA with Dunn's multiple comparisons.*

CONCLUSION:

- Repeated synchrotron X-ray imaging studies are now feasible, enabling novel insights about MCT and surface behaviours in live intact airways to be revealed that cannot be achieved, non-invasively, using any other method.
- The reduction in radiation dose has made longer-term monitoring of MCT possible and will facilitate future studies that can include examination of the effect of potential novel therapeutics for CF across days or months.
- Histological tissue analyses for radiation induced change are underway.

ACKNOWLEDGEMENTS:

SPring-8 proposal 2012A1661.
Experiment Funding: NHMRC, WCH Foundation and Cure4CF Foundation.
Experiment Travel: Australian Synchrotron ISAP.
Conference Travel: Robinson Institute.

SPring-8, Japan.