ADVANCES IN AIRW[illegible] CYSTIC FIBROSIS: EXTENDED MONITORING OF INDIVIDUAL PARTICLE MUCOCILIARY CLEARANCE

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BACKGROUND:

- The CFTR ion channel defect in epithelial cells lining the airways impairs mucociliary transport (MCT).
- Quantification [illegible] 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 magnificat[illegible] / resolution at least two orders [illegible] magnitu[illegible] methods such a[illegible] MCT [illegible]**
- 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 [illegible] changes [illegible] individual animals[illegible]
- **This study examined the [illegible] er particles in the lungs of live mice for up to 2[illegible]**

MET[illegible]:

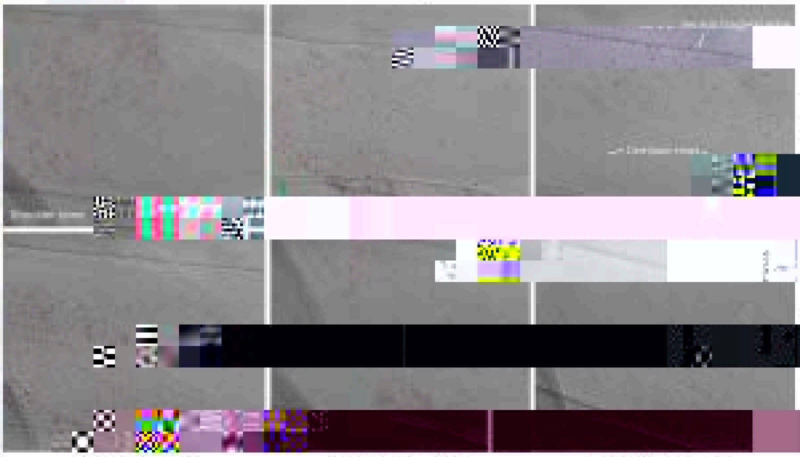
- Intubated (flexiVent[illegible] BL20XU beamlin[illegible]
- Lead marker particles were delivered to the trachea using a Dry Powder Insufflator™ (PennCentury, PA).
- Images of the trachea with an effective pixel size of 0.56 μm and a field [illegible] view of 1.43 mm x 1.2 mm were captured using a high-resolution camera. All images used a 50 ms exposure:
  - Baseline: (12 images 5 sec apart) x 2[illegible] 5 min apart.
  - Repeat: 110 images 5 sec apart.

[illegible] 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 [illegible] was used to [illegible] rates.

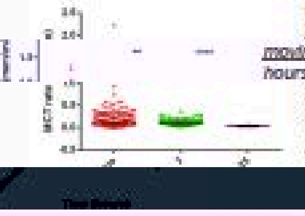
RESULTS:

- The repeat imaging protocol was well tolerated, with no discernable effects from the radiati[illegible]
- Both qualitative and quantitative [illegible] MCT rate was reduced at the late[illegible]



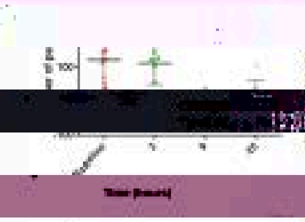
(a) M1: 3 hours (b) M2: 9 hours (c) M3: 25 hours

(F1) High magnification images of lead dust in the trachea of three live mice (M1-M3) after particle insufflation, assessed using a repe[illegible] 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) 25 hours later. The head is to the right and the spine is t[illegible] Imaging included the same bone edge (bottom LH corner) to ensure the same a[illegible] region was examined each time. Imaging location is just above the carina. Stationary parti[illegible] and (the few) moving particles in green (arrows[illegible]

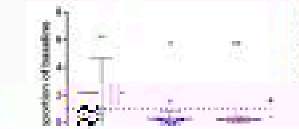


(F2) MCT rate of all moving particles over time. Note that no moving part[illegible] es were detected at 9 hours. [illegible] MCT rate at 9 and 25 hours was significantly reduced compared to baseline

***p<0.01, ****p<0.0001, Kruskal-Wallis One way ANOVA with Dunn's multiple comparisons.*



[illegible] of particles present at 9 and 25 hours was significantly reduced [illegible] Dunn's multiple comparisons.



(F4) Proportion of particles (moving and stationary) at each repeat imaging point, compared to baseline. Significantly less particles remained at 25 hours compared to 3 hours.

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-ter[illegible] ing of MCT possible and will facilitate future studies that can [illegible] the [illegible] effect of potentia[illegible] therapeutics for CF across days or months.
- Histological tissue analyses for radiation-induced changes are underway.

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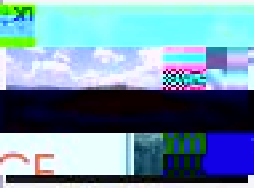

SPring-8, Japan.