DELIVERY DYNAMICS AND DESTINATION OF GENE VECTOR INSTILLATIONS IN LIVE MOUSE AIRWAYS

Martin Donnelley[1,3,4], Karen Siu[5,6], Aidan Jamison[7], David Parsons[1,2,3,4]

1. Respiratory and Sleep Medicine, [illegible] Children's Hospital, Adelaide, South Australia
2. Women's and Children's Health Research Institute, Adelaide, South Australia
3. School of Paediatrics and Reproductive Health, University of Adelaide, South Australia
4. Centre for Stem Cell Research,
5. School of Physics, 6. Monash Centre for Synchrotron Science, Monash University
7. Division of Biological Engineering, Monash University

BACKGROUND: Most groups use the mouse nose as an *in vivo* model site for developing gene therapies for CF airway disease. Reliable dosing of an airway pre-treatment followed by a lentiviral vector is essential for [illegible] standardised delivery techniques we see variability in reporter-gene histological assessments and electrophysiological measurements. The aim of this experiment was to use synchrotron X-ray imaging at SPring-8 to accurately determine the fate of fluids delivered into live mouse nasal airways and the trachea.

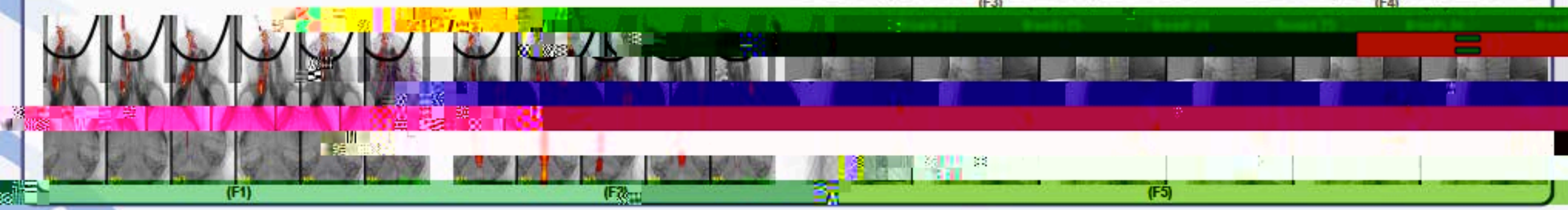
METHODS: Nembutal anesthetized BALB/c mice (n = 6 nasal & n = 4 trachea) were imaged on the BL20B2 beamline at the SPring-8 synchrotron (Japan). For the **nasal study** images were captured at 1Hz. After 1 min of baseline a 4 µl sample of iodine-based contrast fluid (airway pre-treatment surrogate) was delivered over 10 [illegible] 20 µl bolus (gene-vector surrogate) was delivered over [illegible] imaging continued for a further 10 min. Fluid motion was revealed using a background subtraction method. For the **tracheal study** mice were intubated and ventilated at 80 breaths/min with 1 image captured per breath. After 1 min baseline a 15 µl bolus (airway pre-treatment or gene-vector surrogate) was delivered over 30 sec. Following [illegible] min of data collection an additional 15 µl bolus was delivered over 5 sec. Image capture continued for a further 10 min. Frame differencing was used to reveal fluid motion.

NASAL AIRWAY RESULTS:

- The **4 µl dose (F1)** was retained on the treated side, supporting the strength of gene expression seen in nasal ciliated epithelium [illegible] validating the use of a 4 µl dose to retain an [illegible] untreated nasal airway as a with [illegible]
- Confirms why gene expression is limited to the treated [illegible] extends into the tubular nasopharynx [illegible]
- Although the dose also distributes into the lateral transitional/olfactory region, expression from the gene vector was restricted to respiratory epithelium in our gene [illegible]
- The **20 µl dose (F2)** overwhelms the 'holding capacity' of the dosed nostril, with some fluid continuing into the trachea and likely into deeper lung airways.
- Supports our findings of long term lung expression using [illegible] and suggests small gene vector doses may produce the [illegible]
- Supports the proposed [illegible] of action since we only observe gene [illegible] areas reached by the 4 µl dose, despite demonstrating the wide reach of the 20 µl dose.

TRACHEA RESULTS:

- Substantial dose losses may occur upon delivery as [illegible]
- Speed of bolus [illegible] targeting of conducting [illegible]
- Retrograde fluid motion (F3)
- The fast fluid bolus delivery created larger localized increases in the contrast fluid volume that were more easily visualized (F4) using this technique.
- A bolus of fluid, marked with a red X, can still clearly be seen moving down the left main [illegible] and deep lung.



CONCLUSION: Synchrotron imaging can help explain the mechanisms underlying published outcomes from our gene-transduction protocols in mouse nasal airways. Our findings suggest the need for, and permit, much greater attention to dose [illegible] orientation, volume, speed – and enable improvements in dosing technique design.

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