INDIVIDUAL PARTICULATE MUCOCILIARY TRANSIT ANALYSIS USING SYNCHROTRON X-RAY IMAGING

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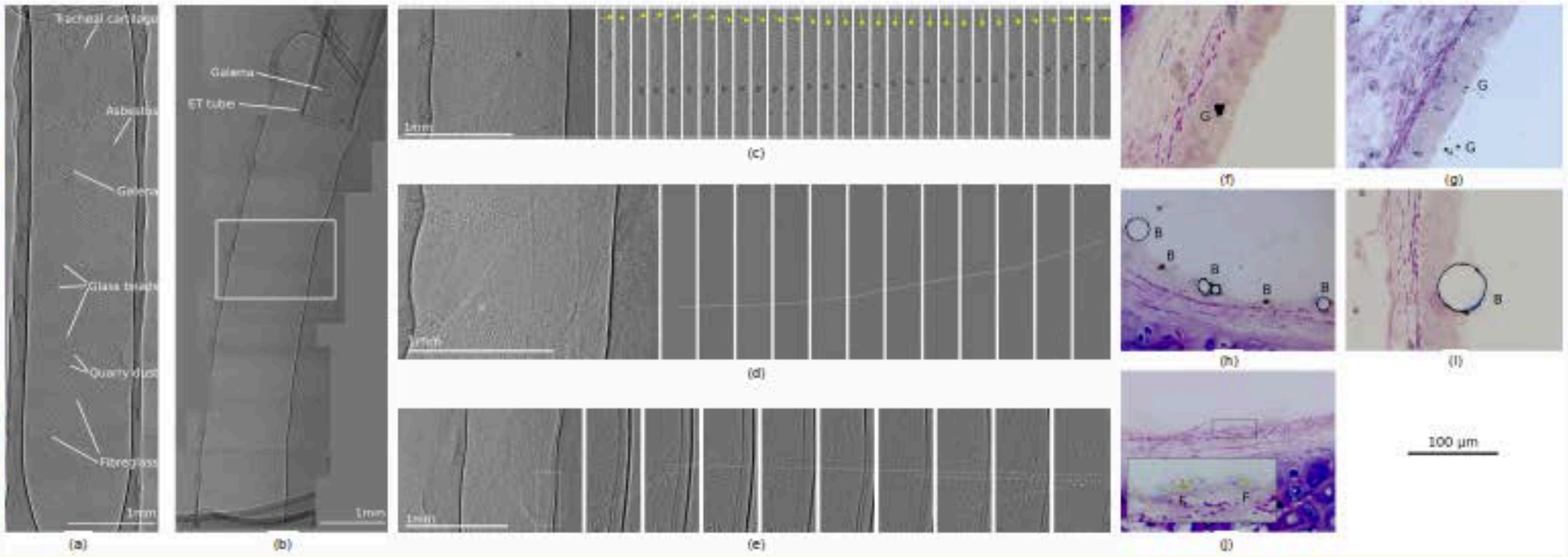
BACKGROUND: The ability of the airways to move and clear deposited particles is a clear diagnostic indicator of airway health. Prior to the development of synchrotron phase contrast X-ray imaging (PCXI) non-invasive detection and tracking of individual particle movements produced by mucociliary transit (MCT) was not possible. Since MCT is affected in cystic fibrosis, the ability to quantify MCT in CF disease models could assist in examining airway surface dysfunction and responses to treatment. Using PCXI we have begun to dynamically and non-invasively examined the behaviour of individual pollutant particles deposited on the airway surfaces of live intact mice to document particle visibility and behaviour.

MATERIALS AND METHODS: Experiments were performed on the BL20XU beamline at the SPring-8 synchrotron. Asbestos, fibreglass, quarry dust, galena lead ore, and reference hollow silver-coated glass beads were examined. One HOS:HR-1 mouse was humanely killed, the trachea excised, and particulate delivery was tested *ex-vivo*. *In-vivo* behaviour was then examined in anaesthetised mice (n=15), which were secured head-high on an imaging board before the X-ray beam was directed laterally through the trachea. 15µl doses of 1% w/v particulates in saline were delivered to the trachea via an oral ET tube. Post-experiment the mice were humanely killed and the trachea was excised, fixed in PFC-OsO_4, embedded in resin and examined with light microscopy and TEM.

RESULTS: All particles were visible *ex-vivo*, but asbestos was not visible in *in-vivo* experiments. (a) *Ex-vivo* results showing the appearance of all particulates. (b) The ET tube and imaging region. (c) Galena clump swirling *in-vivo* during transit. (d) Small quarry dust particle moved up the trachea by MCT. (e) Hollow glass beads near the dorsal tracheal wall. Galena particles (f) in the epithelium and (g) trapped in surface mucus. Distinctive glass beads (h) on the airway surface and (i) displaced into the airway epithelium. (j) Small fibreglass fibres embedded in the overlying mucus.



DISCUSSION AND CONCLUSION: Particle behaviour was related to both the type and size of the particles. Smaller particles moved faster and for longer periods, and we speculate this was because they took longer to be captured on the epithelial surface or in mucus. The transit of all particles was consistently localised to the dorsal tracheal wall. Histological analysis showed that galena particles and glass beads displaced cilia and surface liquid to lodge into the airway epithelium. This experiment demonstrated that PCXI provides the unique ability to non-invasively detect and track individual particulates in live airways, and that PCXI techniques are now a valuable addition to the suite of imaging tools available for use in live airway models.

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