Medical Bio EX Proposal Report

Proposal number:

2008B1985

Title of experiment:

Dynamic synchrotron x-ray detection of individual pollutant particle behaviour after deposition onto live airways

Name and affiliation of the project leader:

Dr David Parsons, Women's and Children's Hospital, North Adelaide, South Australia

Beamline used:

BL20XU Downstream

Research purpose and background

Increasingly, the role of particulate pollution on airway and lung health is being investigated to understand how inhaled particles are dealt with by the respiratory system. Particulate matter (PM) consists of very small liquid and solid particles floating in the air, along with various gaseous pollutants. Types of PM include building dusts (e.g. asbestos, fibreglass) wind-blown dusts, and coal or mineral dusts from mines and quarries. Industrial and motor vehicle pollution is important in urban areas, as is smoke from wood-burning stoves and from cooking fires.

The ability of the airways to move and clear inhaled particles via mucociliary transport (MCT) is a clear diagnostic indicator of airway health. The ability to measure this clearance activity in chronic diseases such as cystic fibrosis and asthma, or to assess environmental hazards from pollutant particles that can be inhaled, is of clear benefit. However, respiratory medicine and science currently lacks a non-invasive method for detecting such particles or for tracking their transport, retention or clearance along airways.

We have demonstrated the ability to locate, track and quantify the motion of introduced hollow glass beads to measure MCT, but in our 2008B experiments we extended this research using more common inhaled particles, relevant to the detection and ultimately the prevention of respiratory disease in both children and adults. We tested the reliability, accuracy and applicability of phase contrast X-ray imaging (PCXI) for the non-invasive quantification of MCT of several different particles. Our aims were to determine the detectability of particles representing different physical characteristics *in-vitro*, and to establish the spatial and temporal resolution of PCXI for tracking these particles during MCT along live mouse airways.

Experimental/analytical method

The experiment was performed in the downstream BL20XU hutch. The imaging and experimental setups are described in two publications [1, 2]. The experiment consisted of an *in-vitro* study to determine which of a range of biologically-relevant particulates were detectable using PCXI. The *in-vivo* behaviour of the detectable particles was examined after deposition onto live mouse airways.

A range of potentially respirable pollutant particles were examined dry or suspended in a carrier fluid (distilled water) to determine their visibility using PCXI. Chrysotile (white asbestos) and fibreglass from a commercial pipe insulation were separately ground under water to produce fibres suited for

examination. Galena — lead sulphide — and dolomite quarry dust were also tested. Other relevant compounds included hollow silver coated glass beads, graphite, titanium dioxide, tintacarb, tin, aluminium, standard roadside PM10 and PM5 particulate matter, nanogold, printer toner and combusted diesel particles. Small samples were placed onto the exposed adhesive surface of short lengths of adhesive Kapton tape and the particles were sealed in place using a second piece of tape. The samples were mounted on a controllable X-Y stage in the hutch for imaging.

For the *in-vivo* experiments 10 hairless mice (Crlj:CD1-*Foxn1*^{nu}) ~18-26 grams were imaged. Mice were anaesthetised with Nembutal and were humanely killed via Nembutal overdose at the end of each study without awakening. The anaesthetised mice were secured on a polyethylene imaging board containing an open slot to allow the passage of the X-ray beam directed anterior to posterior along the mouse centre line. A distinctive bone suture line in the nasopharyngeal airway posterior to the olfactory region provided an easily locatable imaging landmark. Nasal airways were utilised since they are commonly used as a model site for pre-clinical testing of airway function after gene transfer procedures by our group [3] and others [4]. This region is also well suited to these first live PCXI particulate imaging procedures because the airway is stable within the head and shows little movement during breathing; in contrast, lung airways are in almost continuous motion during breathing.

Images were captured at 0.2Hz. After one minute of baseline collection, samples of particulates suspended in distilled water were delivered via a nasal cannula either manually or using a syringe pump, at volumes between 15 µl and 50 µl, in one bolus over 10 to 60 seconds (see Results). Image collection was continued at 0.2Hz for a further 19 minutes, creating a dataset consisting of 240 images.

Research results

At a propagation distance of 135 cm, many particles were visible, however the largely carbon based particulates – combusted diesel, PM10 and laser printer toner – were not. We chose particulates that were visible, and most relevant, for testing *in-vivo*: asbestos, fiberglass, quarry dust, lead and our reference hollow glass beads (see Fig 1).

Based on the *in-vitro* studies, an initial testing protocol for use in live mice was created to achieve maximal use of each mouse and the limited beam time available. After a brief baseline imaging period (no treatment) each animal received one of the fibrous particle instillations (asbestos or fibreglass), along with the reference hollow glass beads. Approximately 25 minutes later one of the non-fibrous particulates (galena or quarry dust) was delivered manually over 10 seconds. This protocol ensured that all particulates could be tested using a small number of mice, while the simultaneous delivery of the control particles (glass beads) was used to confirm that successful instillation had occurred. Based on the overall particulate density seen in the *in-vitro* PCXI images, concentrations of 0.5% w/v for fibreglass and asbestos (mixed with 0.5% w/v reference glass beads), and 0.1% for galena and quarry dust were chosen. We found that a 15 µl dose volume provided sufficient visible fluid and particulates to be monitored in the nasal airway, but induced only a brief increase (< 1 min) in respiratory effort. Importantly, subsequent dosing in the same animal was also well tolerated with this dose-volume.

All five particulates could be detected *in-vivo*, although with different degrees of visibility and with varying levels of difficulty. On the airway surface the fibrous particulates were surrounded with fluid; since this was of similar refractive index to these particles it resulted in a reduction in phase contrast and a loss of particle visibility within the fluid envelope. The rate at which the different particulate types moved along the airway varied widely, between 0.1 mm/min and 4.4 mm/min.

Fig 2 shows that the appearance of asbestos fibres as revealed using motion-detection software. Individual asbestos fibres were not detected, but appeared as agglomerations of enmeshed fibres trapped in liquid. The enveloped structure could be seen moving along the airway. We noted that there

were few of these agglomerations present, but where detected they were large compared to the sizes of the individual fibres, in some cases up to 1 mm in length. The quarry dust was also readily detectable *in-vivo (*Fig 3) where it appeared as compact and irregularly-shaped particles. The smallest detectable dust particles were approximately 10 µm. Although fibreglass was substantially less visible than either asbestos or quarry dust *in-vitro*, it was readily detectable *in-vivo* (see Fig 4). Fibreglass fibres displayed the smooth, enveloped, but irregularly shaped form similar to that seen with asbestos. Despite its high density galena was difficult to detect *in-vivo* (not shown). The hollow glass beads (not shown) appeared as individual particles and their appearance did not indicate a liquid envelope was present.

In summary, this project established the smallest sizes of the different types of particles that we can detect; examined how different types of particles moved along the airways; and established the potential of this new non-invasive imaging method to assist in research into the initiation of childhood and other respiratory diseases that are related to particulate inhalations.

Current and future issues/challenges

The aerodynamic diameter of inhaled particulates must be less than 10 µm to be inhaled into the lung of humans. An improvement in the minimum size detectable under PCXI will be important for ensuring biological relevance in future instillation or inhalation studies. The lead-containing particles were ground galena which is a form of lead different to that inhaled as lead fumes (e.g. lead sulphates or oxides) from a lead smelter, or in leaded petrol, and the pipe lagging fibreglass may not be representative of that producing exposure at health-affecting levels in industrial or residential settings. Although convenient experimentally, delivering particles into mouse airways within a distilled water bolus is not usual in life; inhalation as an aerosol or a dry powder is more physiologically relevant, and planned studies will examine this particle delivery approach. While the skull-bone suture line is easy to locate in the mouse nasal airway and produces a repeatable and stable imaging location under PCXI, we are now (2009A) examining effects in lung airways since this is the relevant target organ.

In these first studies little effort was made to limit radiation dose, which was too intense to consider animal recovery and repeated imaging. The current rapid development of synchrotron and imaging technology suggest there will continue to be improvements in CCD sensor technology, and analytical techniques such as motion-detection and particle tracking. Combined, they should lead to improved image resolution and higher light sensitivity, allowing smaller — and therefore more physiologically relevant — particulates to be detected and tracked.

References

[1] D. W. Parsons, K. Morgan, M. Donnelley, A. Fouras, J. Crosbie, I. Williams, R. C. Boucher, K. Uesugi, N. Yagi, and K. K. W. Siu, "High-resolution visualization of airspace structures in intact mice via synchrotron phase-contrast X-ray imaging (PCXI)," *Journal of Anatomy*, vol. 213, pp. 217-227, Aug 2008.

[2] M. Donnelley, K. Morgan, A. Fouras, W. Skinner, K. Uesugi, N. Yagi, K. Siu, and D. Parsons, "Real-time non-invasive detection of inhalable particulates delivered into live mouse airways," *Journal of Synchrotron Radiation*, vol. 16, 2009.

[3] M. Limberis, D. S. Anson, M. Fuller, and D. W. Parsons, "Recovery of airway cystic fibrosis transmembrane conductance regulator function in mice with cystic fibrosis after single-dose lentivirus-mediated gene transfer (vol 13, pg 1961, 2002)," *Human Gene Therapy*, vol. 13, pp. 2112-2112, Nov 2002.

[4] B. R. Grubb and R. C. Boucher, "Pathophysiology of gene-targeted mouse models for cystic fibrosis," *Physiological Reviews*, vol. 79, pp. S193-214, 1999.

Status of publication and patent

From this experiment we have published article number 2 in the references above.

Keywords and annotations

particles; non-invasive; airway surface; synchrotron radiation; X-ray phase-contrast; asbestos; mouse; detection; X-ray imaging; radiography

Figs

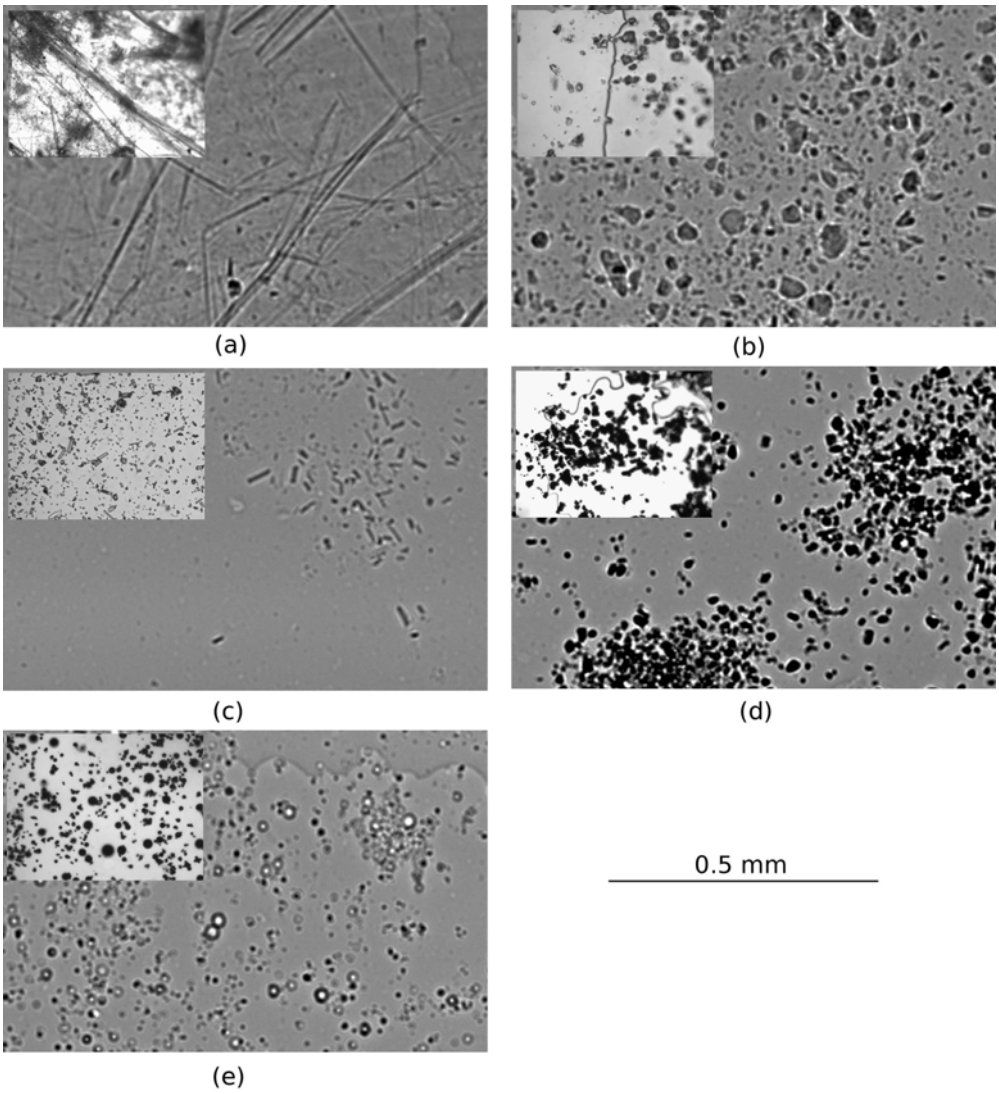

Figure 1 *In-vitro* dry particulate testing revealed the particulates that were visible using PCXI. Samples were examined under PCXI (main pictures) and light microscope (insets): (a) asbestos, (b) quarry dust, (c) fibreglass, (d) galena, and (e) silver coated hollow glass beads. The morphology of each of the particulates is clearly very different. For layout and presentation purposes, and so they are at the same level of magnification, the PCXI images are cropped to 0.90 mm x 0.60 mm, and the light microscope images are 0.365mm x 0.27mm.

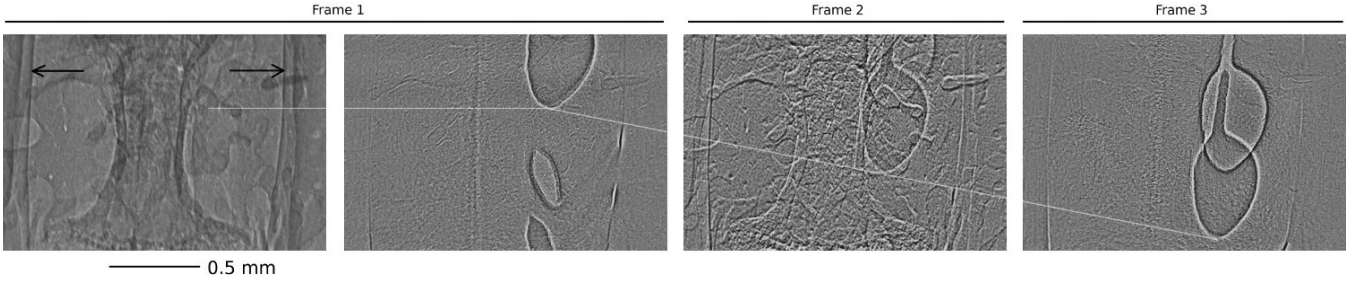

Figure 2 Asbestos particulate detection. The two panes on the left show the original PCXI image and its corresponding motion-detected frame that revealed the moving object on the airway. The panes on the right are the next two motion-detected frames in the sequence. The black arrows mark the nasal airway edge running vertically through the image, and the white lines follow the same object across the three sequential frames each separated by 5 seconds. The last frame shows an enveloped clump of fibres with an elongated tail. These enveloped structures moved along the airway faster than all other particles studied, here at a rate of 4.4 mm/min.

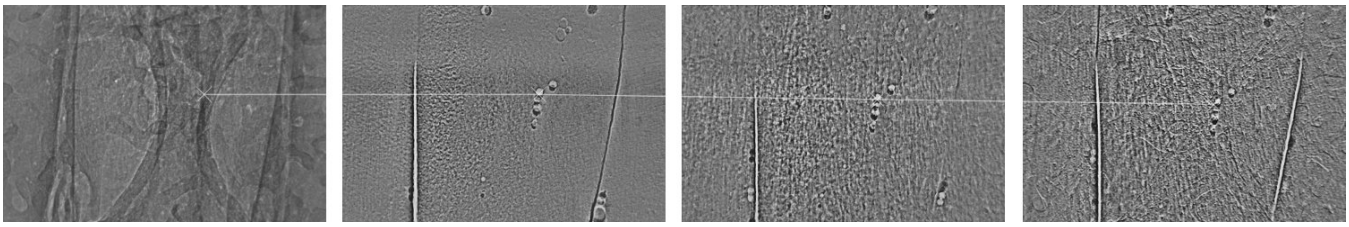

Figure 3 Four particles of quarry dust are visible at the centre of the motion-detected frames. Particles did not aggregate or have the appearance of trapping liquid around them on the airway surface. In this sequence the quarry dust particles are moving at a rate of 0.41 mm/min.

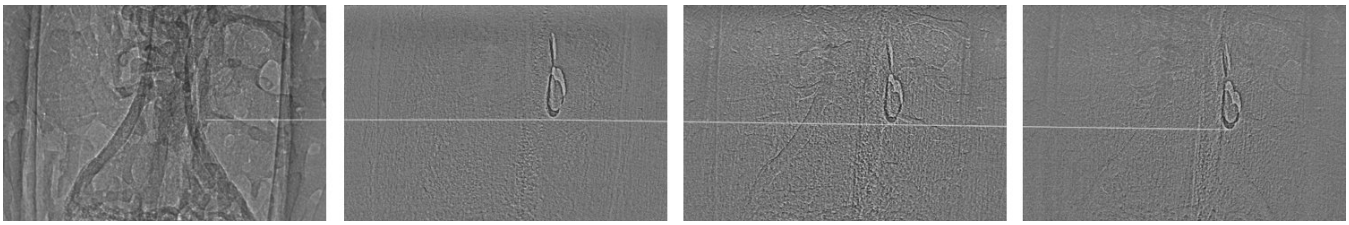

Figure 4 Fibreglass appears similar to asbestos, but the objects were smaller and had shorter tails. Like asbestos, the fibres appeared to smoothly trap liquid around them so that individual fibreglass fibres could not be seen. Fibreglass moved at the same speed as the quarry dust, at 0.41 mm/min.