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Application for beam time at ESRF – Experimental Method

Proposal Summary

When it is inhaled, asbestos triggers a chain of events that can lead to several lung diseases, such as **asbestosis**, **pleural mesothelioma**, an aggressive cancer of the lung lining, and **lung cancer**. The mechanism by which asbestos causes cancer has been intensively studied, but, despite the large and increasing incidence of respiratory/pulmonary diseases due to occupational exposure to asbestos, **a clear understanding of the carcinogenesis is still far to be achieved**. A deeper knowledge of the interaction between asbestos fibers and the organic tissue can lead scientists in their effort to develop more effective medical treatments. **The proposed experiment aims to reveal the location and distribution of asbestos fibers in the alveolar network of untreated human lungs samples, and their association with the biological tissue** (alveolar macrophages or giant cells, in particular). This will be achieved by exploiting **high resolution computed microtomography.**

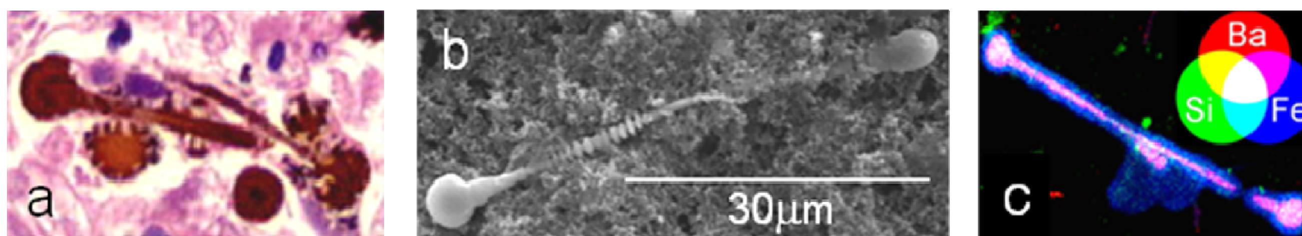

Fig. 1. (a) Optical microscope image of an asbestos fiber (*AB*) embedded in a histological section (400x). (b) Scanning Electron Microscope image of an *AB* extracted from lung tissue. (c) X-ray microfluorescence map (acquired at ID21) showing the co-localization of Si, Ba, and Fe (map size 90 x 65 μm^2, pixel size 0.5 x 0.5 μm^2).

Scientific background:

In the lungs, asbestos and other toxic fibers irritate the tissue, causing minerals and proteins to cluster around the foreign fibers. These clusters are known as ferruginous bodies or asbestos bodies, if the embedded fiber is asbestos (abbreviated as *AB* in the following), and are the product of a bio-mineralization process carried out by alveolar macrophages around the inhaled fibers (Fig. 1a and 1b). It was generally accepted that the coating surroundings the fibers was a protective mechanism deposited by macrophages trying to segregate the cytotoxic fibers from the organic tissues[1]. However, other authors suggested that the coating material itself may enhance the cytotoxic properties of asbestos by increasing the generation of free radicals[2]. These studies also demonstrated that the iron contained in the coating is catalytically active[3] and can induce modification in DNA[4]. Earlier studies[5] suggested that the coating contained crystalline particles of the same order of size of the inorganic iron core of the ferritin molecule. On this basis it was assumed that the crystalline material comprising the major part of the *AB* is composed of ferritin. Scientists converge to the conclusion that the presence of redox-active iron, either as a constituent of the crystal or adsorbed to its surface, is responsible for the genotoxic and cytotoxic effects of amphibole asbestos, and currently, it is widely accepted that the coating consists of protein (probably ferritin) and iron. While it is agreed that amosite and crocidolite are the most hazardous asbestos fiber types, chrysotile, an iron-free form of asbestos, has produced tumors in animals and is a recognized carcinogenic in humans. In addition, the discover of an excess of mortality from mesothelioma in individuals living in three villages in Central Anatolia (Turkey) due to chronic exposure to erionite[6], an iron-free fibrous mineral belonging to the zeolite group, questioned the central role of iron in the pathogenesis. **The majority of the studies on *AB* suffer from the fact that suitable microprobe techniques, which are required to study objects at the micro-scale** (*AB* usually do not exceed 100 μm in length and 1 μm in diameter), **became widespread only recently**. **Bulk techniques,** such as Inductively

Coupled Plasma Mass Spectrometry, **require heavy treatments of the samples** (incineration or digestion in strong acids), which can **alter the chemical composition of the *AB* and remove the organic component.** These techniques also completely **lack of spatial information. Common microprobes such as scanning and transmission electron microscopies** were **widely used** for this subject, but also **require some sample treatments** such as the preparation of **histological section** or **carbon sputtering** on the surface of the samples. In addition, the information that can be obtained by those techniques is **limited to two dimensions. Microprobe tools based on synchrotron radiation have started to be exploited only recently by a single research group** to study the present topic, and **2D phase contrast experiment**, in particular, has been used only once on this topic[7]. **X-ray tomography is a powerful technique that can produce precise 3D images of human tissue.** Unlike conventional x-ray imaging, **phase contrast imaging** relies on the changes in the real part of the refractive x-rays index as they pass through different tissues, and is much more sensitive than conventional absorption x-ray imaging[8].

Experimental technique(s), required set-up(s), measurement strategy, sample details (quantity...etc):

Lung tissue samples from two former asbestos plant workers, **who were affected by lung cancer or pleural mesothelioma**, were collected after forensic autopsy and preserved in formalin (10%). The authorization to perform research on the samples was obtained by two local bioethical committees. Non-neoplastic portions of lung tissue were examined to estimate the number of *AB* per gram of dry weight (g_{dw}) by optical microscopy and scanning electron microscopy, following the procedure described in Belluso et al.[9] In both specimens the burden of *AB* ($\sim 3.6 \cdot 10^5$ and $\sim 1.2 \cdot 10^6$) largely exceeded the amount established to indicate a high level of occupational exposure to asbestos ($1 \cdot 10^3/g_{dw}$)[10]. **Ten lung portions from each of the two 10x10x10 mm^3 histological specimens** will be brought to the ESRF in sealed plastic cylindrical containers of size compatible with the experimental setup and analyzed without further treatments (the samples can be cut in smaller pieces to fit with the detector field of view). Propagation-based phase-contrast CT imaging set-up will be used. The incident working energy will be 26 or 35 keV, to reduce dose effects; the sample to detector distance will be set to maximize the contrast (~0.1-0.3 m depending on resolution and chosen energy). To lower the total acquisition time, lower resolution (1-2 μm) setup (10x) will be used to measure large sample volumes and to locate regions of the samples rich in *AB;* then higher resolution setup (40x) will be used to measure these regions at higher resolution (~0.5 μm).

Beamline(s) and beam time requested with justification :

The ESRF is a world leading laboratory for the application of phase-contrast imaging[11] and ID19 would be the ideal beamline to perform the proposed experiment given the availability of the necessary flux, setup and instrumentation for high resolution phase-contrast CT. Considering the number of samples (20) and time required to locate the *AB*, at least 9 shifts will be necessary to complete the experiment. As a second option, the experiment could be done at ID17 even if this beamline is not optimized for such high resolution.

Results expected and their significance in the respective field of research:

CT of untreated bulk lung samples (i.e. preserving the 3D alveolar structure) impacted with toxic fibers has never been attempted before, and will reveal important information on the exact location and distribution of the fibers and on their interaction with the organic tissue with very high spatial resolution. Our research group has acquired high resolution 2D μXRF maps on asbestos bodies extracted from human lungs (Fig. 1c and exp. report MD550), which revealed their elemental distribution with unprecedented level of detail (Fig1c). A proposal to perform μXRF tomography will also be submitted to ID16B-NA beamline, and the information acquired will complement x-ray computed tomography, furnishing an exhaustive characterization of *AB* in lung tissue samples. Data will be analyzed following the phase retrieval approach.

References

[1]A B Kane *et al*. Human Path. 34, 735 (2003); [2]H Pezerat *et al.* Toxic. and Ind. Health 8, 77 (1992); [3]A J Ghio *et al.* Toxic. Path. 32, 643 (2004); [4]L G Lund *et al.*, Occupat. & Environ. Medicin 51, 200 (1994); [5]F D Pooley. Environ. Res. 5, 363 (1972); [6]I. Baris, Int. J. Cancer 39, 10 (1987); [7]L Pascolo *et al.* Particle & Fibre Toxic. 8, 7 (2011); [8]A Bravin *et al.*, Phys. Med. Biol. 58 R1 (2013); [9] E Belluso *et al.*, Microch. Acta 155, 95 (2006); [10]P De Vuyst, Europ. resp. J. 11, 1416 (1998); [11]Y Zhao *et al.* Proc. Nat. Ac. Sci. 109, 18290 (2012).