Asbestos bodies in human lung tissue studied with synchrotron radiation induced fluorescence and phase contrast tomography

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Occupational exposure to asbestos is universally associated with several lung injuries, including respiratory diseases, asbestosis, mesothelioma, and, owing to others co-factors, lung cancer. Pleural malignant mesothelioma, in particular, is highly deadly, the 5-year survival rate being of 5% only (1 over 20). In addition, due to weathering of asbestos-reinforced cement products, asbestos contamination is also becoming of concern for the general population, in particular in urban areas. Asbestos can enter in living organisms by inhalation and, due to its high biopersistence, can manifest its toxicity after 20 to 60 years. For these reasons, although since the 1990s asbestos started to be banned in most countries and it is almost abolished today, it remains a current major worldwide health threat. In fact, it is foreseen that the peak of mesothelioma cases in the world will be reached within 2020 [1]. Once inhaled, asbestos irritates the tissues causing minerals and proteins to cluster around it. These clusters, which assume very peculiar shapes (Figure 1), are known as asbestos bodies (*ABs)*, and are the product of a biomineralization process carried out by alveolar macrophages. It was generally accepted that the coating surroundings the fibers was a protective mechanism set up by macrophages in the attempt to segregate the cytotoxic fibers from the biological tissues [2]. However, other authors suggested that the coating material itself might enhance the cytotoxic properties of asbestos by increasing the generation of free radicals [3]. These studies also demonstrated that the iron contained in the coating is catalytically active [4] and can induce modification in the DNA [5]. Earlier studies [6] suggested that the coating contained crystalline particles of the same order of size of the inorganic iron core of the ferritin molecule, which has been later identified with ferrihydrite, a poorly crystalline iron oxide. Today, scientists converge to the conclusion that the presence of redox-active iron, either as a constituent of the asbestos crystalline structure, or adsorbed to its surface, is responsible for the genotoxic and cytotoxic effects of amphibole asbestos. Nevertheless, precise knowledge of the coating composition and formation mechanism prevents formulating solid hypothesis on the carcinogenetic mechanism. Asbestos bodies usually do not exceed 20-80 µm in length and 1-6 µm in diameter. The majority of the studies on *ABs* suffer from the fact that suitable microprobe techniques required to study very small objects became widespread only recently. Electron microscopy techniques (SEM and TEM), require aggressive sample preparation, which usually include the removal of the biological tissue by attack with strong acids, or cutting the samples in thin or ultrathin sections. Furthermore, these techniques, as standard laboratory analyses, are performed on histological sections, thus on quasi bidimensional samples. On the other hand, x-ray tomography, and, in particular, phase-contrast x-ray tomography, which adds the information of the phase contrast to the conventional absorption contrast, can natively reveal the tridimensional information on bulk biological samples, without the need of invasive sample preparation procedures. In this work, the innovative combination of x-ray fluorescence and phase-contrast tomography, both operated at nanoscopic resolution and exploiting synchrotron radiation, allowed revealing the elemental composition and distribution, the density, and the mass of single *ABs* embedded in the original lung tissue of former workers of a fiber-cement plant, who have been exposed to continuous and massive contamination to asbestos.

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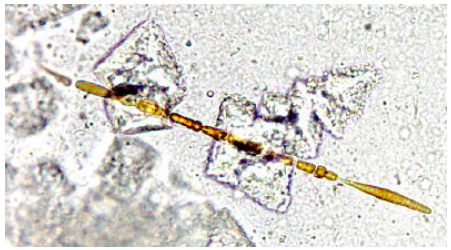

Figure 1a. Optical microscopy image of an *AB* recovered from lung tissue by chemical digestion (400x).

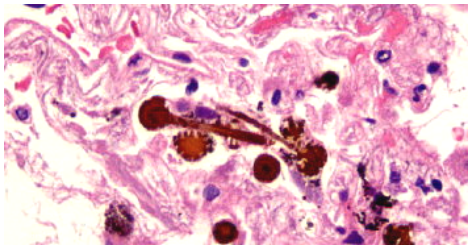

Figure 1b. Optical microscopy image of *ABs* in a 3µm-thick histological section stained with haematoxylin and eosin (400x).

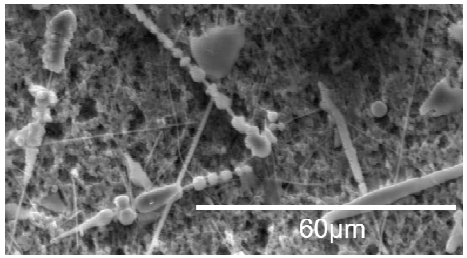

Figure 1c. Secondary electrons SEM micrograph of *ABs* recovered from lung tissue by chemical digestion.

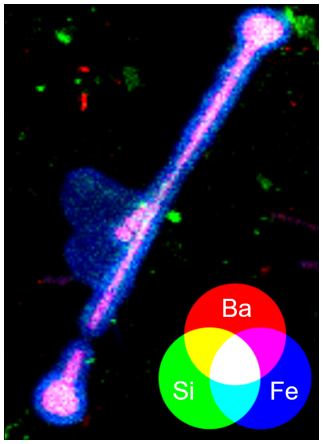

Figure 1d. [7] Distribution and co-localization of Si, Fe, and Ba in an *AB* obtained by induces x-ray fluorescence (incident x-ray energy 7.3 keV, pixel size of 0.5 x 0.5 µm^2).