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Nano-imaging of environmental dust in human lung tissue by soft and hard X-ray fluorescence microscopy☆

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ABSTRACT

It is well recognized that a large number of pulmonary diseases are induced by the effects of inhaled particulates. Anthracosis is defined as an asymptomatic, mild form of pneumoconiosis caused by the accumulation of "black carbon" in the lungs due to repeated exposure to air pollution or inhalation of smoke or coal dust particles. Since the human population is progressively exposed to an increasing number and doses of anthropogenic micro and nano particles/compounds, there is a pressing urgency to explore toxicological impact arising from these exposures and the molecular mechanisms driving the body defense or possible related diseases. The toxicity mechanisms are clearly related to chemical composition and physical and surface properties of materials. A combination of synchrotron radiation-based (SR-based) nano X-ray fluorescence (XRF) imaging and soft X-ray microscopy was used to chemically characterize environmental particulates (anthracosis) in lung tissues from urban subjects with the aim of better understanding the complex nature of related lungs' deposits. High-resolution XRF analyses performed at ESRF and Elettra synchrotrons allowed discriminating single particles in the heterogeneous aggregates found in the lung tissue. The small particles have variable composition resulting from the different combinations of Ti with O, K and Si, Al and Si, or Zn and Fe with O. Interestingly, simultaneous absorption and phase contrast images showed the particulate morphology and allowed to predict the presence of very dense nanoparticles or high concentration of heavy elements.

1. Introduction

Lung diseases are among the most common medical conditions in the world [1,2]. Tens of millions of people suffer from them due to infections, genetics predisposition and exposure to smoking or inhalants.

Pneumoconiosis is a group of occupational lung diseases caused by inhaled dust particles and fibres, which causes inflammation of the pulmonary parenchyma leading to fibrosis, affecting the airways or alveoli. Among these, coal workers' pneumoconiosis, also known as black lung disease, is caused by long exposure to coal dust, and commonly affects coal miners and others who work with coal. The initial, milder form of the disease is called anthracosis [3–8], an asymptomatic accumulation of black pigment in the lung tissue and in the related lymph nodes, which can be found in varying degrees among most urban dwellers and in tobacco smokers. Air pollution is unavoidable nowadays and consists of a complex mixture of different components with possible synergic toxicological and carcinogenic effects [3,9–14]. Exposure to mineral and organic particulate, especially in the form of fine particles, may induce genetic damage and has been related to an increased risk of cancer in humans [15].

It is problematic to assess the pathogenicity of poorly soluble materials in the form of dust particles and fibres, since their effects are determined not only by the chemical composition but also by their physical properties and biopersistance. Additionally, inside the tissues dust particles and fibres may undergo complex metabolic transformations and their surface may be modified by removal and deposition of chemical elements, metals, and salts, or by adsorption of macromolecules such as proteins [6,16].

Despite the fact that there are many reports and studies on air

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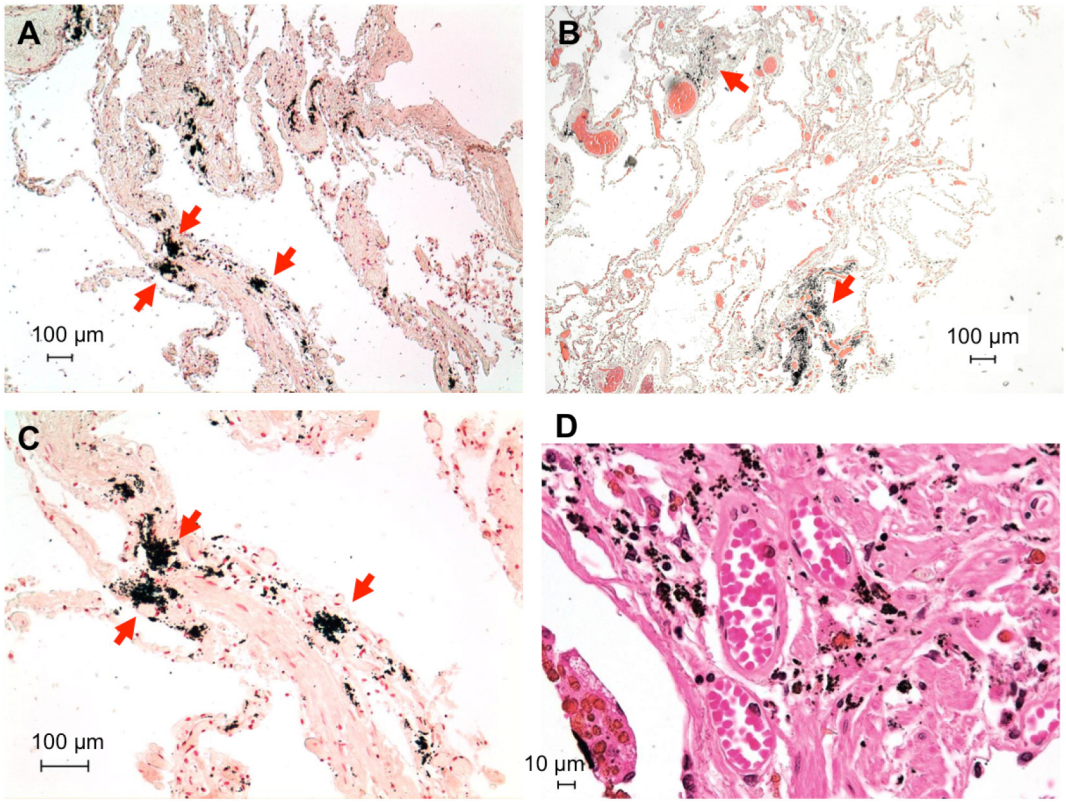

Fig. 1. Photos of hematoxylin and eosin (H&E) stained lung tissues from three different patients (A–C, B and D respectively) and at different magnifications (Panels A and B 10×, Panel C 20× and Panel D 50×) highlighting the presence of black-pigmented deposits in different lung tissue areas: in interlobular septa (A, C), around bronchovascular bundles (B) and in the fibrous tissue (D). Panel C is a higher magnification of Panel A. Scale bar are 100 μm for Panels A, B, C and 10 μm for Panel D.

particulate matter (PM) characterization [17–23] from different part of the world, to our knowledge only a very few sparse examples of lung tissue chemical analyses are reported in literature [6,16,24–28]. There have been a few attempts with SEM and TEM-EDS [29]: although the spatial resolution of these techniques is clearly higher than XRF, the more complicated sample preparation and their lower detection sensitivity limit the information that can be gained from their results [30].

The aim of this work is to apply Synchrotron Radiation X-Ray Fluorescence (SR-μ XRF) spectromicroscopy [31–34] to track the environmental dust particles or fibres inside histological samples of lung tissue and to investigate the chemical nature at nanometer spatial resolution.

In this work, the chemical nature of anthracotic material, found in lung tissues from undefined environmental exposure, was investigated by means of a sequential use of two synchrotron beamlines, ID16B-NA and TwinMic, at two different facilities, ESRF (Grenoble, France) and Elettra Sincrotrone Trieste (Trieste, Italy).

2. Materials and methods

2.1. Patients and sample preparation

Human lung samples were derived from post-mortem examination of three patients which were selected from the archive files of the Unit of Pathology of CRO of Aviano (Italy) [6,16]. The patients had lived in urban centers of the region Friuli Venezia Giulia without known specific professional exposure. Human samples consisted of tissues discarded after forensic autopsy, and were retrieved with the approval of the institution. The identification of anthracosis was performed by light microscopy (DM2500, Leica Microsystems, Germany) on 3–5 μm thick sections from paraffin-embedded samples of non-neoplastic lung tissue both unstained and stained with hematoxylin and eosin (H&E) according to standard protocols. For X-ray imaging and XRF analyses, the unstained 5 μm thick sections were mounted on ultralene foils (4 μm thick) and air-dried, as previously described [16,35,36].

2.2. Synchrotron-based nano X-ray fluorescence analyses

The synchrotron XRF experiments have been carried out at two different synchrotron facilities. In both experiments, the X-ray beam was focused on the sample through suitable X-ray optics and the sample was raster scanned across the beam. For each pixel in the raster scan the X-ray Fluorescence (XRF) was collected by energy dispersive silicon drift detectors (SDD).

The samples were firstly analysed at the ID16B-NA beamline [37] of the European Synchrotron Radiation Facility (ESRF, Grenoble, France) where the pink ($\Delta E/E = 0.01$) 17.54 keV X-ray beam was focused by Kirkpatrick-Baez (KB) mirrors to a spot size of 60 nm × 60 nm on the sample plane (photon flux $4 * 10^{10}$ photons/s). The XRF emitted by the sample were collected by two SGX sensortech SDD arrays, each with an active silicon area of 80 mm^2, using an acquisition time of 100 ms per pixel in the raster scan. A standard reference material from NIST (bovine liver SRM 1577B) was measured for calibration of the X-ray Fluorescence spectra to get semi-quantitative results on some transition metals. Spectra were fitted based on the configuration derived from the measured standard using PyMCA [38] for quantification of the elemental content. The obtained concentrations are expressed in g/g assuming a 1.5 μm thick protein matrix with density 1.2.

Further XRF analyses were carried out on the previously mentioned specimens at the TwinMic beamline [39] (Elettra - Sincrotrone, Trieste, Italy) under a low energy microscopy set-up. TwinMic microscope was operated in scanning transmission mode (STXM) where the beam is focused on the sample through a zone plate diffractive optics (600 μm in diameter with 50 nm outermost zone width) providing sub-micron