PhD Project Proposal

XXXIX cycle

Project Title:

Elongated mineral particles in biological fluids: a possible key for modelling their *in vivo* behaviour

PhD candidate: Alice Marconato

E-mail: alice.marconato@uniroma1.it

Suggested internal tutor: Prof. Paolo Ballirano

E-mail: paolo.ballirano@uniroma1.it

Suggested external tutor: Prof.ssa Violetta Borelli (Università degli Studi di Trieste)

1. INTRODUCTION AND STATE OF THE ART

Asbestos fibres are easy to inhale through airways to pulmonary alveoli. Lung carcinoma (LC) and malignant pleural mesothelioma (MPM) are the two thoracic tumours associated with asbestos exposure (1). Even though asbestos production and use have been banned in Italy since 1992, many contaminated sites are still present, and many ex-exposed workers are still at risk of developing cancer due to the long latency period. Furthermore, ban and regulations cannot be applied to natural occurrences of asbestos (NOA), where weathering events and/or anthropic activities (2) may disturb NOA-bearing outcrops, thus provoking the formation of potentially inhalable fibres.

In recent years attention encompassed other types of elongated mineral particles (EMPs) (3) that may share similar geometry with asbestos, while differing in mineralogy, such as erionite (4) and antigorite (2), whose exposure has been linked to high incidence of LC and MPM (5,6). More than 400 mineral species occur under an elongated habit, and they are widespread in Italy. In detail, NOA have been observed in at least 10 Italian regions (7).

The mechanisms of asbestos-induced carcinogenesis are still poorly understood; so far, the scientific community has identified three major parameters determining fibre (geno)toxicity: (i) the fibrous morphology (8), (ii) fibre biodurability (9) and (iii) chemical reactivity, namely the presence of surface iron ions able to generate free radicals by Fenton reactions (10).

A complex chain of physico-chemical transformations and biological reactions occur when a fibre gets in contact with biological materials. In case of highly bio-persistent minerals such as amphibole asbestos, fibres evolve while reacting with body fluids, immune cells and biological surroundings. These modifications can alter the fate of fibres (phagocytosis/internalization, translocation) and their chemical reactivity, thus contributing to their pro-inflammatory and (geno)toxic potential.

Several investigators demonstrated that asbestos fibres could interact with many different proteins affecting either their structure and/or functions (11-13). Conversely, mineral fibres opsonization by proteins such as vitronectin and immunoglobulins modulates key cellular responses in asbestos-induced toxicity: release of superoxide and tumour necrosis factor and internalization/phagocytosis by inflammatory cells (14 -21). Systematic investigations of possible fibre modifications occurring in different simulated lung fluids (SLFs), mimicking lung interstitial and phago-lysosomal compartments, have been performed to predict how fibres may react with their biological surrounding (22). Anyway, the typical formulations for SLFs lack all representative native molecules of the respiratory tract: proteins, proteoglycans, and surfactant lipids. Bronchoalveolar lavage fluid (BALF) obtained for diagnostic purposes, through instillation and subsequent recovery of a saline solution from one or more lung segments, provides useful information about lung alveolar environment (23). BALF contains a broad spectrum of proteins, and in asbestos-exposed individuals it has been shown to contain elevated levels of iron, transferrin, lactoferrin and ferritin (24). Interestingly, ferritin represents the main protein of ferruginous bodies coating (25) and has been shown to bind to asbestos fibres (13).

Although BALF represents the first fluid encountered by EMPs during their passage through the respiratory system, there are no studies addressing the interaction between EMPs and this body fluid.

Furthermore, from the lung asbestos fibres (and erionite) reach the pleura where they produce a variety of responses (pleural plaques, MPM). Interaction of fibres with pleural environment is even more complex and unexplored than that with the lung.

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2. RESEARCH OBJECTIVES

2.1 Overall objective

Understanding the interaction of hazardous (asbestos, erionite) and potentially hazardous (antigorite) mineral fibres with biological fluids (BFs) of the lung and pleural compartments.

2.2 Specific objectives

- Investigation of EMPs biodurability in BFs from the bronchoalveolar and pleural regions.
- Mineralogical characterization of the samples after interaction with BFs.
- Investigation of possible modulation of chemical reactivity of the fibres after interaction with BFs
- Investigation of the ability of protein absorption by the fibres after incubation with the BFs.

3. IMPLICATIONS

The well-established use of lung simulated biological fluids as an *in vitr*o technique to predict the possible *in vivo* behaviour of inhaled elongated mineral particles (EMPs) represents an oversimplification due to the lack of specific components that may affect the dissolution mechanisms and biological activity of inhaled fibres. The systematic investigations of possible EMPs modifications occurring in BFs more likely reflect the *in vivo* behaviour of the materials under investigation. Moreover, the study of fibres of well know carcinogenicity (asbestos and fibrous erionite) can help predict the toxicity and pathogenicity of “unregulated” or unclassified fibres (antigorite).

The proposed innovative approach, which integrates mineralogy and medicine, may be very effective in order to reveal EPMs-induced carcinogenicity, thus offering a strategy for developing specific cancer prevention strategies and therapies.

4. WORK PLAN

During the 3 years research project the planned activities can be divided into five work packages (WP) each of which consists of several activities. During my doctoral project, I expect to gain valuable knowledge about how the biological environment affects the properties of inhaled EMPs (both in terms of surface reactivity and protein absorption), since until now most studies have been focused on the effects caused by EMPs on the body.

WP1. Sampling and biological characterization of BFs (November 2023-July 2024)

- T1.1 Sampling of BFs (provided by Azienda Sanitaria Universitaria Integrata Giuliano Isontina ASUGI, University of Trieste).

Diagnostic BALF from occupationally exposed workers suspected to have asbestos-related diseases. Clinical and occupational data will be extracted from the medical records.

- Study BALF (related to asbestos correlated diseases): pool of BALF from patients with a diagnosis of lung cancer or MPM.
- Control BALF (not related to asbestos correlated diseases): pool of BALF from patients with a diagnosis of non asbestos-related diseases.

Pleural fluid from pleural effusion (PE, which represents the build-up of excess fluid between the layers of the pleura) collected by thoracentesis or thoracoscopy.

- Study PE: asbestos associated exudative effusion
- Control PE: transudative pleural effusion, most often brought on by congestive heart failure.

- T1.2 Biological characterization of BFs supernatants

Biochemical analysis of cell-free BALF and PE supernatants: iron and proteins binding iron or involved in iron homeostasis (ferritin, transferrin, lactoferrin and myeloperoxidase) [by spectrophotometric, enzymatic and Enzyme-Linked Immunosorbent Assay (ELISA) methods].

WP2. Mineralogical characterization of EMPs: asbestos, erionite and antigorite (November 2023-July 2024)

- T2.1 Bulk crystal chemical characterization of EMPs primarily sampled at newly found Italian outcrops, using a well-tested multi-analytical approach (SEM-EDS, TEM, EMPA, Mössbauer, XRPD).
- T2.2 Surface characterization of selected mineral fibres (XPS, TEM)

WP 3. Evaluation of the biodurability and surface modifications of EMPs under incubation with biological and simulated fluids (August 2024-February 2026)

- T3.1 Dissolution experiments in both open (flow-through cells) and closed (Falcon tubes) systems using BFs and SLFs.
- T3.2 Measure of cations release as a function of incubation time by ICP. Quantification of the dissolution rates of the EMPs samples
- T3.3 Investigation of chemical structural modifications undergone by the fibres following dissolution (same methods adopted in WP2).
- T3.4 Modelling of the dissolution process in both BFs.

WP4. Protein fibre absorption and activity of fibre-associated enzymes will be evaluated as previously described (11, 13). **(November 2023-July 2024)**

- T4.1 Evaluation of protein-binding capacity of mineral fibres directly by incubating them with purified iron binding/bearing enzymes/proteins (ferritin, transferrin, lactoferrin and myeloperoxidase) by spectrophotometric or (for protein without enzymatic activity) resolving fibre detached proteins by Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis and identifying them by Western blotting analysis.
- T4.2 Evaluation of protein-binding capacity of mineral fibres by incubating them with previously characterized BFs pools as described in T.4.1

WP 5. Dissemination and acquisition of new skills

- T5.1 Study of the literature.
- T5.2 Attending PhD courses and seminars.
- T5.3 Mobility abroad (about 2 months during the second year)
- T5.4 Dissemination of the project results in conferences/workshops.
- T5.5 Writing of scientific papers.
- T5.6 Writing of PhD Thesis (last 10 months of third year)

5. MILESTONES

Milestones are located at the end of the corresponding WPs.

- Biochemical characterization of proteins involved in iron homeostasis in BALF and PE from patients with asbestos related diseases.
- Mineralogical characterization of EMPs after interaction with the BFs.
- Comparison of EMPs biodurability in BFs.
- Comparison of EMPs chemical reactivity in BFs.
- Integration with protein absorption data obtained on samples after incubation in BFs.

6. DISSEMINATION PLAN

The PhD will result in the publication of several scientific papers in peer-reviewed journals. I will also present my findings by attending national and international conferences including those of associations such as the Italian Society of Mineralogy and Petrology (SIMP), the Italian Geological Society (SGI), the European Geosciences Union (EGU), the International Mineralogical Association (IMA) and the Italian Society of General Pathology (SIP).

7. TRAINING ACTIVITIES

Throughout the three years of my PhD, I will attend the institutional courses of the Doctoral Program in Earth Sciences at the Sapienza University of Rome, seminars organized by the Sapienza University, University of Trieste, Elettra Sincrotrone Trieste and other scientific institutions. Other activities may be added during the doctoral program.

8. MOBILITY ABROAD

To develop my skills in the field of the various Electron Microscopy techniques that are of paramount importance for a detailed description of structural details EPMs, I plan to spend my mobility period

at laboratory of prof. Jerrold Abraham (Departments of Pathology, State University of New York, Syracuse, NY, USA), to learn the Ion Microscopy methodology.

9. GANTT CHART