PhD proposal 4: Analysis of the changes induced by asbestos fibers on the structure of absorbed proteins and lung tissue architecture

1. Consortium

- University awarding the degree: University of Trieste, PhD in Nanotechnology
  Proposed supervisor: Dr. Lisa Vaccari
  Proposed academic supervisor: Dr. Bernareggi Annalisa

- Proposing CERIC partner facility: Elettra Sincrotrone Trieste, SISSI- Chemical and Life Sciences branch, Trieste, Basovizza, Italy
  Lead proponent: Dr. Lisa Vaccari

- Contributing CERIC Partner facilities: Ruđer Bošković Institute (RBI), Particle-Inducted X-ray Emission and Rutherford Backscattering, Zagreb, Croatia
  Lead collaborator: Dr. Iva Božičević Mihalić

- Contributing CERIC Partner facilities: National Institute of Materials Physics (NIMP), HRTEM facilities, Bucharest-Magurele,Romania
  Lead collaborator: Dr. Corneliu Ghica

- Contributing CERIC Partner facilities: Elettra Sincrotrone Trieste, TwinMic beamline, Trieste, Basovizza, Italy
  Lead collaborator: Prof. Dr. Gianoncelli Alessandra

2. Scientific background

Occupational exposure to asbestos is associated with severe lung injuries such as asbestosis, pleural mesotelioma and lung cancer (Env Health Persp 88, 319-22; 1990). A peak of mortality of many thousands of exposed subjects is predicted for the next 10 years. Understanding the mechanisms of interaction of the asbestos fibers with the cells is mandatory for clarifying in detail the pathogenetic pathways of these diseases and for setting up a therapeutic approach for treating exposed subjects.

Up to now, the mechanisms of fiber entry into the cells and in the various subcellular compartments, of the cytotoxicity of the fibers, of the interaction with membrane proteins and of the effects induced by these events on cell physiology (production of proinflammatory factors, apoptosis) are only some of the topics which wait to be fully understood. A key process is anyway the interaction of the fiber with proteins: this can trigger their entry into the cytosol and the nucleus, can induced cytoskeleton modification and programmed cell death.

Identifying the proteins capable of binding to the fiber and the modifications induced in these proteins by the binding itself should be compelling and mandatory for designing the complete pathogenetic scenario. Many proteins are able to bind to the asbestos fibers, including ferritin (J Toxicol Env Health 52: 343-352,1997) RNA-binding proteins, cytoskeletal proteins, ribosomal proteins (Cancer Science 102: 2118-2125), enzymes (chymase, b-hexosaminidase, eosinophil-peroxidase) (Int J Environmental Res and Public health 15: 104, 2018). The association "enzymatic protein-fiber" can induce a significant increment of the enzymatic activity, which may be possibly induced by a structural modification of the absorbed protein. This possibility is also in agreement with the finding that fiber-associated ferritin in the so called "asbestos body" (AB, a fiber coated with ferritin and other components) changes its secondary structure

(from α-helix rich to β-sheet rich) (J Toxicol Environ Health A 2007). These peculiar structures are used to confirm the exposure for legal purposes, but accordingly to recent reports can also exert a cytotoxic activity, possibly mediated by the abundant catalitically active Fe^{+3} (Scientific Reports 7:44862, 2017) and/or the acquisition of peroxidase activity (J Toxicol Environ Health A, 75 (11), 603-23 2012).

On the basis of the findings on the enzymatic activity and ferritin secondary structure, we put forward the hypothesis that ***the protein binding to the asbestos fiber is the causal event which can trigger structural changes in the protein and modify its function (increment of enzyme activity, misfolding of ferritin).***

3. Outline of the experimental protocol

We plane to study in detail the nature of specific proteins structural changes following the absorption on asbestos fibers by exploiting two different experimental models, as follows:

1. Pure proteins will be incubated with different amount of various types of asbestos fibers containing iron (crocidolite and amosite) and iron-free/ low-iron containing (chrysotile), for different times, at different pH and in the presence of different Ca^{2+} concentrations. Control incubations will be carried out using non-asbestos like-fibers (wollastonite and TiO2) and in the absence of fibers. We will first consider (apo)ferritin and subsequently pure enzymes with a pro-inflammatory role, such as chymase, and leukocyte peroxidases. Cell extracts containing various enzyme activities will be also processed. The amount of the protein absorbed on the fibers will be evaluated with adequate methods. The secondary structure of fiber-bound proteins will be analyzed by FITR. The specific protein function and features will be also evaluated (enzyme activities, ferroxidase activity of apoferritin and UV-spectral changes) and compared with control fiber associated proteins (if any) and native proteins. The secondary structure modification of fiber-bound proteins will be investigated by infrared nanoscopy (SISSI-BOFF@Elettra), while the fiber modifications by HR-TEM combined with EDX analysis (NIMP). The fibers will be deposited on appropriate substrates and confined in graphene cells for safety purposes. The results achieved with the CERIC-ERIC internal project RENEWALS will be exploited to this aim.
2. Analysis of tissue paraffinated lung sections (already available to the research team) derived either from a murine model (mice instilled intratracheally with crocidolite or saline and sacrificed after one or 6 months; described in: Toxicology letters 241: 111-120; 2016), or from lung biopsies obtained from autoptic samples of exposed subjects deceased with asbestos-related diseases (described in: Scientific Reports 5: 12129, 2015). On these samples, FTIR microscopy, IR nanoscopy via photo-thermal expansion approach (SISSI-BOFF@Elettra), XRF and X-Ray Microscopies (TwinMic beamline@Elettra), HR-TEM (NIMP) and PIXE combined wit RBS imaging (RBI) analyses, and possibly molecular imaging of cells and tissues with MeV SIMS (RBI), will be carried out, with the aim of evaluating at micro and nano-scale morphological, elemental and biochemical profile modifications induced by asbestos fibers, and potentially identify transport mechanisms of abstesos fibers.

4.The expected impact of the proposed research on the overall quality and capability of CERIC

The Time Since First Exposure (TSFE), also known as 'latency', to asbestos has been calculated to be about 30-40 years for Mesothelioma (Environmental Health, 18:71 2019). The standardized annual mortality rates from pleural mesothelioma in Italy are higher in some Northern Regions, such as Friuli-Venezia-Giulia, where the CERCI-ERIC Partner Facility Elettra is based. Nevertheless, the peak of mesothelioma incidence is expected for 2025 in those countries that banned asbestos before 2000, such as Italy or Poland. For the ones that banned it from 2001 to 2012, such as Croatia, Czech Republic,

Romania, or for the ones where it is still not banned, the peak of incidence is expected later. It is therefore clear that asbestos-related cancers will have a significant impact on Central-Eastern Europe Countries in the coming decades.

Finding that the nature of the protein binding to specific asbestos fibers can induce structural changes, which can trigger its functional change as a consequence, will explain at least one step of the AB formation and, more importantly, will open a broader study on various specific proteins with a pro-inflammatory and/or pro-tumoral role, the functions of which may be increased or inhibited by the fiber association. Studying different type of fibers, will also help to highlight the role played by the fiber composition on triggering protein misfolding and consequent fiber toxicity. These studies, carried out at AB level, requires nano-resolved approaches, sensitive to morphology, chemistry and elemental composition, and will be carried out by HR-TEM and nano-FTIR at SISSI-Bio, directly exploiting one of the implementations planned in the CERIC-ERIC INTEGRA project.
Once highlighted the behavior of the association protein-fiber, their localization at tissue level, the chemical modification of the surrounding tissues as well as the dyshomeostasis induced at elemental level will be first investigated exploiting micro-resolved techniques, such as FTIR microscopy, XRM and LE-XRF (as already described by the proponents in Scientific Reports 5: 12129, 2015 and Toxicology letters 241: 111-120; 2016), further exploiting the complementarity of PIXE combined with RBS imaging.
The proposed project deals with a relevant problem for the Countries of the CERIC-ERIC consortium, and crosses the molecular and cell/tissue pillars of the INTEGRA project. It takes advantage from several CERIC-ERIC facilities, proving the synergic complementarity of the analytical tools offered by CERIC-ERIC in terms of type of information and spatial resolution for studying and improving knowledge in Life Sciences.

5. **Estimated cost, if relevant** (tuition fee, salary/stipend, travel)

The following table gives a summary of the expenses per year

Year	tuition fee	Admin. Cost	salary	10% *	stay abroad¥	mobility†	Tot per year
1st	cost of the student	€ 1,800.00	€ 18,850.00				€ 20,650.00
2nd	cost of the student		€ 18,850.00	€ 1,534.00	€ 3,141.33	€ 600.00	€ 24,125.33
3rd	cost of the student		€ 18,850.00	€ 1,534.00	€ 3,141.33	€ 600.00	€ 24,125.33
Total							€ 68,900.66
* Funds for the research activity of the PhD student, per regulation							
¥ Funds for 3-months abroad (€ 2,356.00) are the minimum imposed per regulation. Considering the transnational and multidisciplinary nature of the proposed PhD, we ask for 4-months abroad at the second and 4-months at the third PhD year. In case of unspent budget, this will be re-funded to CERIC-ERIC by the University.							
† Funds for conferences and schools, per regulation							

Annex 1

CURRICULUM VITAE LISA VACCARI

PERSONAL INFORMATION

Name, Surname Lisa, Vaccari
Affiliation
Present Position Elettra Sincrotrone Trieste, Basovizza, Trieste
Head of SISSI beamline, Chemical and Life Sciences branch
Coordinator of IDEAS beamlines
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Nazionalità/Nationality Italian

WORK EXPERIENCE

2006 to now Responsible for the Chemical and Life sciences branch of SISSI beamline (Synchrotron Infrared Source for Spectroscopy and Imaging) at Elettra-Sincrotrone Trieste
FTIR microspectroscopy in the Mid-IR regime for several applications: medicine, biology, biochemistry, chemistry, cultural heritage, forensic science and others.
Users' support.
Beamline maintenance and upgrade.
Budget administration.
Beamline activities and personnel coordination.
Industrial activities: Cooperation with ILO@Elettra (Industrial Liaison Office) for applied research and industrial applications of FTIR microspectroscopy.

2019 to now Coordinator of the beamline group IDEAS: Imaging, Diffraction, Emission Absorption and Scattering beamlines
Management and operational coordination of personnel and budget of the Elettra beamlines belonging to IDEAS

EDUCATION AND TRAINING

1993-1999 MSc in Chemistry
Thesis title: Characterization of cytochrome c2 from Rhodopseudomonas palustris
Supervisor: Prof. Mario Calligaris
Institution: Chemistry department of Trieste University
1999-2002 Research Fellow
Micro- and nano-technology fellowships
Supervisor: Prof. Enzo Mario di Fabrizio
Institution: National Institute for Matter Physics, LILIT (Laboratory for Interdisciplinary LIThography), at Elettra, Trieste, Italy
2002-2005 PhD in Pharmaceutical Science
Thesis title: Strategies for the targeted delivery of doxorubicin in the chemotherapy of colon adenocarcinoma: from micro to nanotechnology
Supervisor: Prof. Maurizio Prato
Institution: Pharmaceutical Science department of Trieste University

RESEARCH ACTIVITIES

- FTIR microspectroscopy of biological systems under physiological conditions.

Lisa Vaccari is expert in bio-spectroscopy and pioneered the exploitation of microfabrication capabilities for the design and fabrication of IR-suitable microfluidic devices for performing in vitro bio-experiments under physiological conditions in real-time.

- Complementation of FTIR microspectroscopy with diverse analytical techniques (AFM, micro-XRF and X-ray Imaging, flow-cytometry, …) for biological system characterization and analysis

- Toxicology of nanomaterials: FTIR microscopy based approaches for nanotoxicity assessment1,2
- Fertility and sterility: FTIR spectroscopy and microscopy for the study of human gametes
- Graphene for life sciences

Project coordination
PI of InCIMa and InCIMa4 projects funded by the European Regional Development Fund and Interreg V-A Italy-Austria 2014-2020 (project website: http://www.elettra.eu/Prj/InCIMa/; www.InCIMa4.eu)

PI Deputy of the CERIC-ERIC internal project RENEWALS (project website: http://www.ceric-eric.eu/index.php?n=Research.Renewals)

Coordinator of Industrial projects with Bio-High-Tech Companies

TEACHING ACTIVITIES
Trieste University, Chemistry Department, Master Student Class: "Tecniche di caratterizzazione con Luce di Sincrotrone"
Trieste University, PhD School in nanotechnology, PhD Student Class: "Microscopies for nanotechnology"

ADDITIONAL INFORMATION
Member of the Synchrotron Peer Review Committee of:
ALS (Advanced Light Source, Lawrence Berkeley National laboratory, san Francisco, USA)
SESAME (Amman, Jordan)
CLS (Canadian Light Source, Saskatoon, Canada)

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CURRICULUM VITAE BERNAREGGI ANNALISA

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Present position ASSISTANT PROFESSOR
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RESEARCH EXPERIENCE AND ACADEMIC POSITIONS

2008 to now Department of life sciences
University of Trieste, Italy
Assistant professor in Physiology, academic discipline bio/09 – physiology

September 2018 A. I. Virtanen Institute for Molecular Sciences
University of Eastern Finland, Kuopio Visiting professor

EDUCATION AND POST GRADUATE TRAINING

February 2008 – March 2008 Department of Neurobiology and Behavior
University of California Irvine, USA Visiting researcher
December 2007 – November 2008 Department of Neurobiology and Behavior
University of California Irvine, USA Visiting researcher
July 2003 – November 2007 Department of Physiology and Pathology
University of Trieste, Italy Post-Doc position

2003 University of Trieste, Italy
PhD in Neuroscience
1999 University of Trieste, Italy
Master's Degree in Biological Sciences
TEACHING EXPERIENCE - 2000-2020: Biophysics (Human Physiology, School of Medicine)
- 2006-2008: Cell Physiology (Master of Biotechnology)
- 2008-2020: Physiology (School of Pharmacy)
- 2009-2020: Principle of Synaptic Transmission (International Master in Neuroscience)
Member of the teaching program of the International Master Degree in Neuroscience (University of Trieste)
RESEARCH ACTIVITY - Mechanisms involved in neuromuscular plasticity and skeletal muscle regeneration.
- Microtransplantation of cell membrane into Xenopus oocytes for the characterization of neuroreceptors.
- Xenopus oocytes as model for studying the asbestos - cell membrane interactions.

MENTORING Supervisor of Master Thesis (University of Trieste, Italy): International Master Degree in Neuroscience, School of Pharmacy, Biotechnology, Biological Sciences and Genomic.
Supervisor of PhD Thesis Neuroscience and Cognitive Sciences (University of Trieste).
PHD EXTERNAL REVIEWER Neuroscience (S.I.S.S.A., Italy), Experimental and Clinic Physiology (University of Varese, Italy), Behavioral Neuroscience (Sapienza University, Rome, Italy), School in Biomedicine and Neuroscience (University of Palermo, Italy)

ONGOING GRANT 2018-2021: "Microgravity-induced gene expression in a nerve-muscle coculture model"
JOURNAL REVIEWER European Biophysics Journal, Purinergic Signaling, Scientific Reports, Peer J., Cellular and Molecular Life Sciences, Neuroscience, Translational Psychiatry, Nanotoxicology.

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