Biomedical Studies

Teresa Pinheiro

The aims of the Biomedical Studies group are the study of putative biomarkers in order to characterize exposure, diseases and therapy efficacy and to identify potential targets for novel therapies.

Efforts were developed in the translation of basic biomedical research into novel diagnostics and therapies for the benefit of human populations exposed to metals, and of patients with chronic diseases.

Undertaken research is an end product of intense and interactive collaborative work among researchers in Cardiology, Pneumology, Dermatology, Biology, Biochemistry, Chemistry and Environmental Sciences.

Current projects join different groups from three ITN Units, Reactor, UCQR and UFA, which are working in consortium with other research institutes, academia and hospitals.

Major research areas focused:

1) Environmental health research establishing new biomarkers of exposure;

2) Clinical outcomes research establishing disease progression and clinical response to therapy;

Recently an interdisciplinary project has been initiated in collaboration with the Universidad Autónoma, Madrid and University of Singapore that will explore safety issues related to nanoparticles. Stem cells will be used as a model, and different techniques will be applied to locate nanoparticles in cells and evaluate the biological response.

Other issues such as bio-availability of metals in aquatic environment are recently being explored under collaboration with the Instituto Nacional de Recursos Biológicos, illustrating the continuous potential of microbeam techniques in life science research.

A variety of scientific and technical skills developed in Biomedical Studies group of ITN, involving proton microscopy, inductively coupled plasma mass spectrometry (ICP-MS), flow cytometry and other cell function evaluation techniques, helped consolidating the scientific niche and launching new areas of research.

Continued funding in the areas of environmental and biomedical sciences during the last years had strengthened existing skills and promoted advanced training of Ph.D. and M.Sc. students.

The main achievements of 2010 are summarised in the following pages.

Research Team
Researchers

T. PINHEIRO, Aux., Group Leader
L.C. ALVES, Aux. (25%)
R. VELOSO, Post-Doctoral, FCT grant

Students

P. NAPOLEÃO, Ph.D., BI grant
B. BATISTA, M.Sc., QREN grant
C. FRANCO, M.Sc., BI grant
C. RAMOS, M.Sc., BI grant
P. FÉLIX, M.Sc. BI, ITN
S. ANDRADE, M.Sc. Student, FC-UL
S. VELOSO, M.Sc. Student, FC-UL

Technical Personnel

R. PINHEIRO

Collaborators

A.B. ALMEIDA, Full Prof., FM-UL/Hospital Sta. Maria
A. BARREIROS, Aux. Resarcher, LNEG
A.M. VIEGAS-CRESPO, Princ. Researcher, FC-UL
F. ARAÚJO, Princ. Researcher, UCQR, ITN
M.C. MONTEIRO, Ass. Prof., CESPU, Porto
M. MOTA CARMO, Ass. Prof., FCM-UNL
M. SELAS, Nurse, CHLC/EPE, Hospital Sta. Marta
P. FILIPE, Aux. Prof., FM-UL/Hospital Sta. Maria
R. CRUZ FERREIRA, M.D., CHLC/EPE, Hospital Sta. Marta
M.D. YNSA, Researcher, CMAM, Univ. Autónoma Madrid, Spain

Exhaled Breath Condensate – a new biomarker of exposure to metals

P. Félix, C. Franco, L.C. Alves, T. Pinheiro, S.M. Almeida, M.C. Freitas, F. Araújo, M. Santos, A. Barreiros[1], A. Bugalho de Almeida[2], S. Garcia[3],

Objectives: The aim of the project is to investigate whether Exhaled breath Condensate (EBC) can be employed for a better risk assessment of human exposure to metals.
The project is a joint initiative of ITN units *Reactor and Nuclear Safety* and *Physics and Accelerators*. The project was carried also in tight collaboration with the *Environmental and Analytical Chemistry Group* of the UCQR/ITN.

Methods: Approximately 100 workers have been enrolled in the study, working at two Pb processing industries located in the Lisbon geographical area. A group of non-exposed voluntaries working in offices was also constituted for baseline interpretation of EBC data. The EBC was collected under tidal breathing conditions using commercial equipment (EcoScreen, Jaeger, Germany) and the elemental concentrations measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) installed at UCQR/ITN.
The particulate matter (PM) in the work place and in offices was evaluated by INAA (URSN/ITN and TUDelft, NL) and PIXE (UFA/ITN). PM was collected in stacked filter units that allowed PM fractioning: 2.5-10 µm in the first stage (PM_{10}) and < 2.5 µm ($PM_{2.5}$) in the second stage.

Results: Implementing standard procedures of EBC collection and analysis for metal exposure assessments was one of the milestones of this project. This objective involved EBC matrix characterization, traceability of the analytical method and EBC collection time optimization. EBC contained a miscellaneous of particles in a variety of dimensions, as can be depicted in Fig 1.

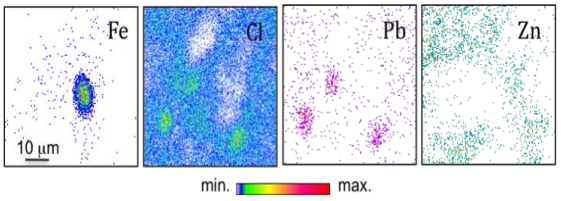

Fig. 1 – Images of the dry deposit of exhaled breath condensate of workers obtained by Proton Microscopy installed at UFA. Particles of varied composition can be identified.

These features influence metal content determination. The study of analytical reliability of EBC showed that acidification was required to obtain sample homogenization. The quantitative results obtained with ICP-MS were validated by comparison to those obtained with Total Reflection X-ray Fluorescence (TXRF).
The EBC collection methodology was examined by studying the variations in EBC metal contents along the working week. In Fig. 2, the Pb levels in EBC are shown. Levels were relatively steady during the working period in Industry 1 and significantly higher than in Industry 2. In the later Pb levels increase in the end of the working week. The EBC of workers showed concentrations well above non-exposed individuals (controls).

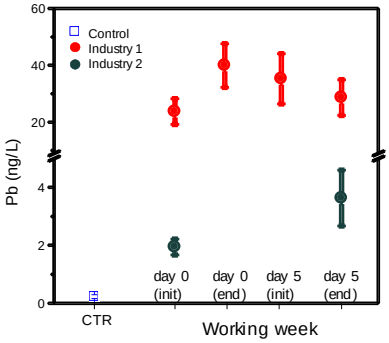

Fig. 2 – Pb concentration in EBC of controls and workers along the working week.

The levels of Pb in airborne particulate matter (PM) in the workplace and in offices compare to Pb contents measured in EBC (Fig. 3).

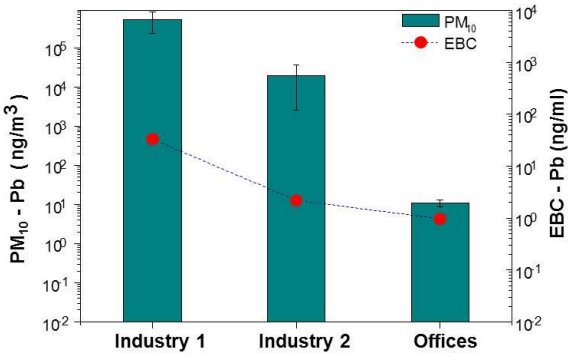

Fig. 3 - Concentration of Pb in PM_{10} collected at each studied site followed Pb concentration in EBC.

Conclusions: 1) It was confirmed that EBC analysis was valid and within limits of confidence <5 % for Cr, Mn, Sb and Pb. **2)** The most representative collection times were at the beginning and at the end of the week-working period. **3)** The elemental concentrations in EBC can be associated to exposure.

Publications

S.M. Almeida, T. Pinheiro, P.M. Felix, C. Franco, M.C. Freitas, L. Alves, A. Barreiros, S.M. Garcia, Int. J. Environ and Health, 4 (2010) 293-304
S.M. Almeida SM, P.M. Felix, C. Franco, et al., Nucl. Instrum Methods A 622 (2010) 453-455
T. Pinheiro, M.A. Barreiros, L.C. Alves et al., Nucl Instrum and Methods B (in press).

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New biomarkers for Coronary Artery Disease

P. Napoleão, C. Ramos*, R. Cruz Ferreira [1], M. Mota Carmo[2], M. Selas [1], M.C. Monteiro[3], M.B. Criado[2], A.M. Viegas-Crespo [4], F. Caeiro [4], A. Turkman[5], V. Andreozzi [5], A.S. Andrade*[4], S. Veloso*[4], T. Pinheiro**

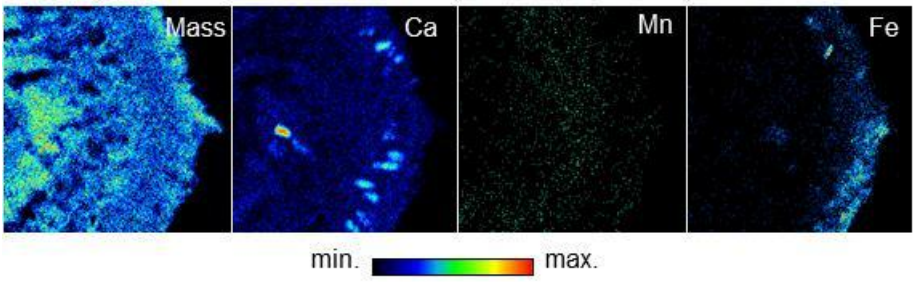
The main goals are the identification and characterization of the vulnerable plaque in hopes of identifying morphologic and physiological features that predict plaque rupture in coronary syndromes. The study is longitudinal, assessing patients to 180 days after intervention. Several molecules, inflammatory cells, and endothelial progenitor cells (EPCs) are been studied. These cells and molecules may have relevant roles in endothelial dysfunction and in the processes involved in plaque rupture as confirmed by the angiographic detection of luminal obstructions and virtual histology intravascular ultrasound (VH-IVUS)-derived measurements of the atherosclerotic plaque. Results obtained so far suggest that variations of several markers (e.g., vascular endothelial growth factor, oxidized lipoproteins, lymphocytes and EPCs) may express the evolution of disease. During 2010 two M.Sc. thesis were carried out under the current projects.

Project funding: FCT/PIC/IC/82734/2007; LAHSM/2010 - Liga de Amigos do Hospital de Sta. Marta.

Metal bio-availability in water-sediment interfaces – a micro-distribution evaluation.

R. Veloso[6], C. Vale[6], T. Pinheiro**

The main objectives are to understand the micro-distribution of trace elements across the interfaces between salt marsh sediments and inhabitant organisms and between sediment and water in natural environment. The micro-distribution of trace elements in sediment profiles were carried out by Proton Microscopy to assess the concentration gradients and infer metal fluxes, specially Mn and Fe, from sediment to the overlying water. Also the elemental profiles between sediments and benthic organisms are being investigated to elucidate whether metals are sorbed on the cellular or tissue wall or uptake by the organism. In the figure below the distribution of Ca, Mn and Fe can be seen in a transversal section of a root. The most external region of the section corresponding to the sediment-water interface is marked by a sharp decrease of Mn and increase of Fe.

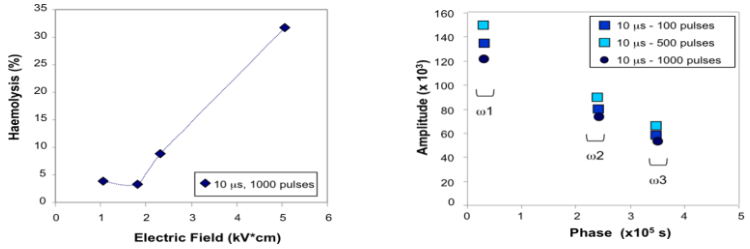


Funding: SFRH/BPD/47473/2008

Effect of Electric Pulsed Field in Human Blood Cells

B. Baptista, V. Dores*, T. Pinheiro*, H. Canacsinh[7], L. Redondo[7]*

The effect of ultra-short pulses (few μs) of high intensity in the permeability of living cell membrane was studied. Human erythrocytes were used to study the electrical model of the cell and calculate the resistivity of the cell membrane. Haemolysis was measured after pulse application. A theoretical mathematical model was developed based on Fast Fourier Transform to extract the amplitude and frequency components of current signals. The graph in the left shows the increase of haemolysis with electric field; the right graph shows the frequency components (ω), which differ according to exposure conditions, illustrating the adequacy of the theoretical model to the cell study. (Collaborative work under the contract QREN -1600-A2P2).



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