Biomedical Studies

Teresa Pinheiro

The activities of the Biomedical Studies group are the study of biomarkers of exposure and disease.

Research activities carried out are 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 national and international research institutes, academia and hospitals.

Major research areas focused:

1) Clinical research targeting chronic diseases;

2) Environmental health problems related with metal exposures;

3) Methodological approaches to assess nanoparticles toxicity.

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, biochemical methods and 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 2011 are summarised in the following pages.

Research Team

Researchers

T. PINHEIRO, Aux.
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Students

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Technical Personnel

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Collaborators

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Endothelial function, inflammation and composition of the atherosclerotic plaque

P. Napoleão[1], C. Ramos, R. Cruz Ferreira[2], M. Mota Carmo[3], M. Selas[2], M.C. Monteiro[4], M.B. Criado[4], A.M. Viegas-Crespo[5], F. Caeiro[5], A. Turkman[6], V. Andreozzi[6], T. Pinheiro

At present, heart disease remains far and away the leading cause of mortality in developed countries. Although some of the traditional risks factors are linked with disease incidents there are no biomarkers for diagnosing coronary artery disease.

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 artery disease (CAD). Several molecules, T lymphocytes and endothelial progenitor cells (EPCs) are been studied, which may have relevant roles in endothelial function and in plaque rupture.

Results

Approximately 80 patients with CAD and 50 controls (CTR) were evaluated. Patients with acute myocardial infarction (AMI), sable (SA) and unstable (UA) angina, were enrolled in the study. Angiographic data of coronaries obtained during angioplasty and percutaneous intervention, and plaque biological characterization obtained with virtual histology intravascular ultrasound (VH-IVUS) are being related with clinical findings and circulating indicators, which may be associated with the disease.

Indicators of the endothelial function and plaque activity, such as vascular endothelial growth factor (VEGF), interleukins, oxidized low-density lipoprotein (ox-LDL), and T lymphocyte activation profile (CD69+ and CD25+), were some of the indicators that are being evaluated and detected in blood by flow cytometry and ELISA.

Results: In CAD patients the plaque content of fibrotic and calcified tissue were correlated with VEGF concentration in serum whereas high levels of ox-LDL were associated with larger plaque areas (PA) as depicted in Fig. 1.

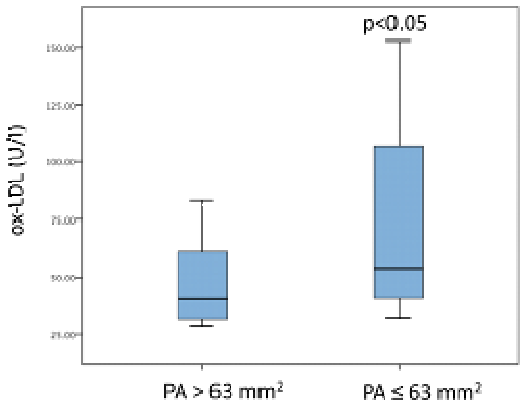

Fig. 1 – Box plots of ox-LDL concentration in two classes (low and high quartiles) of plaque area (PA).

The patients having larger plaque areas and higher levels of ox-LDL in circulation suffered from acute coronary syndromes and the condition was also related to low number of circulating EPCs and high T-cell activation.

CAD patients showed different profiles of T-cells, which express the activation markers CD69 and CD25. In controls T-cells express high CD69 and low CD25 whereas in patients CD69 expression decreased and CD25 augment. In AMI patients CD25- subset expressing high CD69 was most representative and in SA patients the proportion of CD69+ T-cells drastically decreased (Fig. 2). Therefore, the reduced expression of CD69 and activated CD25+ T-lymphocytes may favour immune reactivity of T-cells.

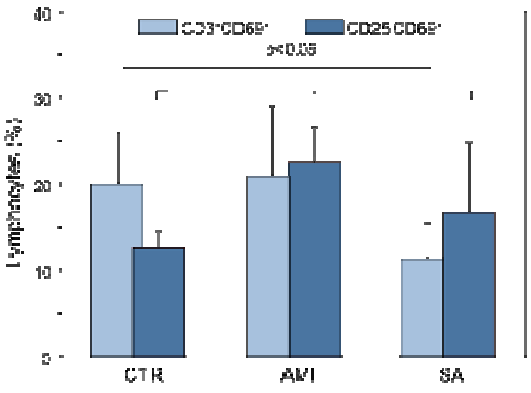

Fig. 2 – T-cells activation profiles in controls and CAD patients.

Conclusions

Circulating markers such as ox-LDL may express plaque vulnerability. The different activation patterns of T-cells in patients and subjects without CAD suggest that T-cell differentiation may condition the immune inflammatory response in these patients. In the overall, changes observed in biochemical indicators EPCs and inflammatory cells were associated to the most adverse disease outcomes.

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[2] Serv. Cardiologia, Hospital Sta. Marta, CHLC;
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Metal toxicity:interaction of nanoparticles with cells

M.D. Ynsa[1], A. Picado[2], A. Barreiros[2], L.C. Alves, T. Pinheiro

The key to understanding the toxicity of nanoparticles (NPs) is that their minute size, smaller than cells and cellular organelles, allows them to penetrate these basic biological structures, disrupting their normal function. Synthetic engineered nanomaterials, which has astonishing physical and chemical properties lead to an exponential use of these materials in multiple aspects of daily life. For example, titanium dioxide (TiO_2) NPs possess photocatalyst activity and are used as antibacterial coatings and in sunscreens. Iron oxide with magnetic properties (Fe_3O_4) is used in clinical diagnostic; quantum dots of CdSe are being explored as molecular probes.
The first aims of our study is to evaluate the toxic effect of NPs of similar equivalent spherical diameter (approximately 10-20 nm) and various elemental compositions on human stem cell lines and living organisms, planktonic crustaceans and aquatic plants. Other goal of our studies is to generate experimental set-up for a cytotoxicity screening of NPs toxicity.
The characterization of NPs is done with scanning electron microscopy and nanoparticle tracking analysis. Nuclear microscopy methods are used to identify NPs in living cells and organisms.
So far, exposure tests were performed in vitro and results on viability, biological response and NPs internalization obtained, though not for all models employed. Mesenchymal stem cells were exposed to functionalized NPs of CdSe and Fe_3O_4 and cell culture viability tests carried out. After 48h incubation with some of the NPs types produced, major morphological alterations were observed in cell cytoskeleton and nuclei. For those that were viable the exposure conditions still need to be tuned. Planktonic crustaceans and aquatic plants were exposed to various concentrations of TiO_2. The NPs distributions in plant tissues from root to leaves were inspected. Major deposits were observed at the external wall in roots and leaves as can be observed in the figure below (Fig. 1). However, minor internalization of NPs in plant tissues was observed.

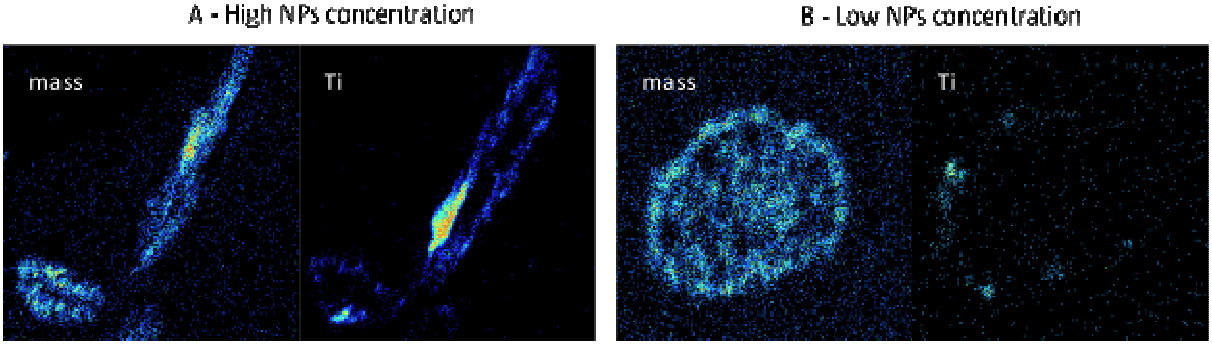

Fig. 1 - Aquatic plant (Lemna) exposed to high and low concentration of NPs of TiO2. Nuclear microscopy images of the mass density and Ti distribution in root sections. NPs deposits were observed at the external wall of roots (Ti maps). A – two sections of roots: longitudinal (centre) and transversal (bottom left) (300x300 μm^2); B – transversal section of the apical root (53x53 μm^2). Rainbow colour scale: minimum content, dark blue and maximum content, red.

[1] CMAM, Univ. Autónoma Madrid; [2] LNEG

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

R. Veloso[1], C. Vale[1], 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, especially 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. The balance of these metals at sediment-water interface are being investigated.

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