Biomedical Studies

Teresa Pinheiro

The research activities during 2008 within the group of Biomedical Studies make use of focused ion beam techniques to image tissue and cell morphology, and other micro-analytical techniques to assess elemental composition and molecular indicators of cell/tissue response.

The fruitful association with researchers within ITN (UQR and Reactor), with researchers outside ITN, of other national laboratories and universities, enabled us to build a true multidisciplinary team.

Also, the instrumental panoply used provides unique analytical capabilities. High-resolution biological imaging with particle beams, elemental analysis with ICP-mass spectrometer, and molecular discrimination with flux cytometry are examples of knowhow developed and combined to reach specific milestones.

Collaborative work carried out, developed skills and the outcome of past research projects paved the way to the present activities.

Ongoing projects focus non-invasive human bio-indicators of pathology and environmental exposure, which can be:

- molecules and microparticles in circulation and body fluids or adhesion molecules, receptors, cytokines, that are expressed in cells to study coronary artery disease;
- Exhaled Breath Condensate (EBC) as a new indicator of exposure to pollutants;
- Nuclear Microscopy and MRI images of Fe in skin and liver in human metabolic disorders;
- images of the atheroma (IVUS-VH - intra-vascular ultrasound virtual histology) associated to specific markers to treat patients with coronary disease in real-time;

Continued funding on areas such as cardiology and environmental health had strengthened existing skills, promoted advanced training for students and created new synergies by involving various research groups from different areas.

The main achievements of the research developed during 2008 are summarised in the following pages.

Researchers

T. PINHEIRO, Aux. (Group Leader)
L.C. ALVES, Aux.

Students

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Technical Personnel

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Collaborators

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Biomarkers in Coronary Syndromes

P. Napoleão[3], R. Cruz Ferreira[1], M. Selas[1], A. Toste[1], M.C. Monteiro[2], A.M. Viegas-Crespo[3], A. Turkman[4], V. Andreozzi[4], R. Carvalho[+], A. Borges[+], L.G. Borges[+] & T. Pinheiro

Objectives

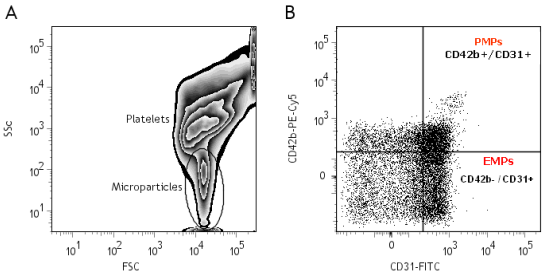
To study new bioindicators of endothelial dysfunction and thrombotic potential in coronary artery disease aside with clinical and biochemical data. Patients with myocardial infarction were assessed at the onset of the thrombotic event previous to intervention and along the recovery period.

Results

Cytokines, soluble and expressed forms of adhesion molecules, and microparticle production were measured. Changes of paramenters measured were monitored over time together with inflammatory markers and specific indicators of myocardial damage. The influence of confounders, such as medication intake, in the measured parameters variations over time was also assessed.

Special attention has been dedicated to microparticles produced by activated endothelial cells, blood cells and platelets in the acute myocardial infarction (AMI).

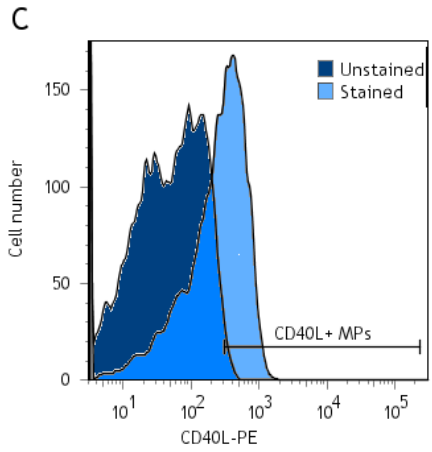
Micropaticles were defined according to morphological characteristics (size and roughness) based on light scattering properties (forward scattering - FSC and side scattering – SSC), and localized below platelets. The representative flow cytometry zebra-plot (contour and density) – A, shows the microparticles region - elliptic area.



In the flow cytometry fluorescence scattergram (B) Platelet microparticles, PMPs (right upper quarter) and endothelial microparticles EMPs (right lower quarter) subsets can be identified using specific labelling antibodies fluorescent in specific fluorescence bands, such as CD31-FITC and CD42b-PE-Cy5.

Fluorescence histograms can be then produced to quantify the positive events of interest. Therefore the expression of membrane-bound molecules such as CD40L, P-selectin, E-selectin, etc. can be quantified.

As exemplified in (C) the surface CD40L-positive-MPs can be selected after unstained sample background set-up. The number of microparticles expressing this molecule can be then estimated in stained samples by counting positive events and measuring fluorescence intensity (CD40L-PE).



The associations found between the microparticle-bound CD40L and CD62E+ with CD40L expression on platelets, on CD4+ and on CD8+ T lymphocytes, at the acute event and in the follow-up, highlighted the complex interplay of CD40L in thrombosis, inflammation and endothelial damage/dysfunction.
The time dependent-variations of soluble CD40L levels and the expression of CD40L on platelets observed in AMI patients, point out differential individual behaviour that may be relevant in AMI prognosis.
In the continuation of these studies, emphasis will be put on apoptosis and plaque macrophagic activity. Assembling all the information collected within the collaborative work, between ITN and Hospital de Santa Marta, will contribute to improve indexes of plaque progression and regression, identification of at-risk patients, and the validation of prognostic value of CD40L in AMI evolution.

Published work

P. Napoleão et al, Serial changes of oxidized-low-density lipoprotein associated with culprit vessel in ST-elevation myocardial infarction – a promising marker? Rev. P. Cardiol. (in press);

P. Napoleão et al, Platelet and endothelial status in AMI. in: 77th EAS Congress European Atherosclerosis Society Proceedings in Ather. Suppl. (in press);

P. Napoleão et al, Oxidized LDL levels associate with culprit vessel in AMI. in: ESC2008 European Society of Cardiology Congress Proceedings (in press).

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[2] CESPU, Porto
[3] Centro de Biologia Ambiental, FC-UL
[4] Centro de Estatística Aplicada, FC-UL
[+] MSc. Students

B – Skin iron as a diagnostic tool in hemochromatosis

T. Pinheiro, L.C. Alves, R. Fleming[1], R. Silva[2], P. Filipe[2], A. Barreiros[3]

The study used conventional and innovative laboratory tests to differentiate distortions of iron metabolism. Patients were genetically characterized and studied before starting and along the phlebotomy therapy. Nuclear microscopy and nuclear resonance techniques provided iron quantitative imaging and physiological information on skin and liver. Biochemical methods provided hepcidin contents in serum and markers of iron metabolism and organ function. There are currently two papers in press.

[1] Hospital de Sta Maria, Serv. Imuno-hemoterapia
[2] Hospital de Sta Maria, Serv. Dermatologia/FM-UL
[3] INETI, Lisboa

C – Exhaled Breath Condensate: A tool for noninvasive evaluation of pollutant exposition?

M. Almeida, T. Pinheiro, A. Bugalho de Almeida[1], S. Garcia[2], A. Barreiros[3],M. F. Araújo, M. Santos, M.C. Freitas, L.C. Alves, P. Félix, C. Franco[4], B. Batista[4]

The aim of the present study is to investigate whether Exhaled Breath Condensate (EBC) can be employed for a better risk assessment among human exposed to toxic pollutants, using industries processing lead. The project is a joint initiative of ITN units UFA and UR, together with the Instituto de Soldadura e Qualidade and the Hospital de Santa Maria. The primary objective is to develop a new non-invasive human bioindicator that could be applicable for professional exposition. During 2008 industries were selected according to the project objectives: number of workers exposed, handled metals, workers shifts, industry localization and industry and workers interest on the project. EBC sampling was tested in voluntaries to establish the better methodology to sample, store and analyse the EBC. Preliminary tests were also made in workers along the working shift to define the most representative sampling time.
EBC chemical characterization are being performed by TXRF in INETI and by ICP-MS in ITN, the latest recently installed under the Programa Nacional de Re-Equipamento. This collaborative work with the Environmental and Analytical Chemistry Group of ITN and LAAQ/INETI will enable methodology validation and perform inter-laboratory exercise for analytical performance comparison.

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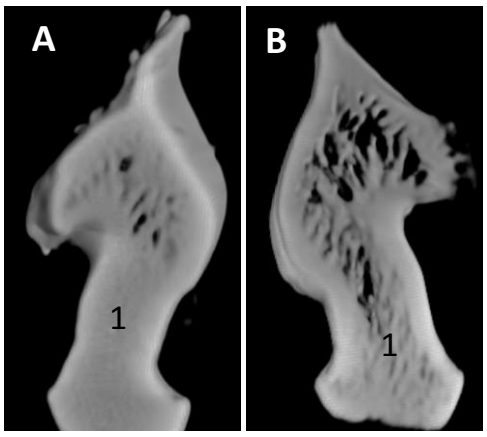
[2] Inst. de Soldadura e Qualidade
[3] INETI, Lisboa
[4] Centro de Biologia Ambiental, FC-UL

D –Nuclear Microscopy and X-ray micro-computed tomography

I. Gomez-Mollila[1], M.D Ynsa[2],L.C. Alves, T. Pinheiro

X-ray micro-computed tomography (µCT) is an excellent tool to examine the morphology of a sample in a non-destructive way, making its inner structure visible.
Rendered reconstructed data of a sample taken from an healthy (A) and an osteoporotic (B) bone slice can be depicted in figure. The osteoporotic sample presents a higher degree of porosity (trabecular bone – 2) in comparison to the healthy bone.



Nuclear microscopy provides quantitative information about the elemental distribution and concentration. Both can be used as complementary techniques in order to get more information about the samples. Osteoporosis will be the major biomedical application.
In order to achieve conclusive quantitative results on densities, a monochromatic source has been used for µCT studies. For this, a project has already been approved at HARWI II beam line, Hasylab DESY: *Density changes in femoral bones from rats under osteoporosis preventive treatments.*

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[2] CMM, Univ. Autónoma de Madrid