Nuclear Microscopy Studies of Skin Permeability to Nanoparticles

T. Pinheiro, L.C. Alves, A. Veríssimo, F. Marujo, R. Silva[1], J.N. Silva[1], P. Filipe[1], M. Amin[2]

Objectives

Nanoparticles of TiO_2 and ZnO are widely used in commercial sunscreens by their capacity to scatter UV wavelengths of sunlight. Skin exposure to commercial products containing nanoparticles of Ti, Zn, and Si oxides, among others and their trans-epidermal diffusion has been studied using nuclear microscopy techniques. One of the objectives of the work is to assess the percutaneous penetration depth of Ti oxides. Therefore, methodologies were adjusted to enable the validation of elemental distribution maps or profiles with high-resolution images originated in transmission mode (STIM, Scanning Transmission Ion Microscopy).

Results

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The permeability of mammalian skin (pig and human) to TiO2 particles (<20nm) is being studied. Several emulsions containing micronised Ti were tested in pig skin. As far as human skin is concerned, three commercial products and one of the test emulsions containing micronised Ti were studied.

Skin is a stratified tissue and the precise identification of skin layers is needed to ascertain the penetration depth of the physical filter from the formulation.

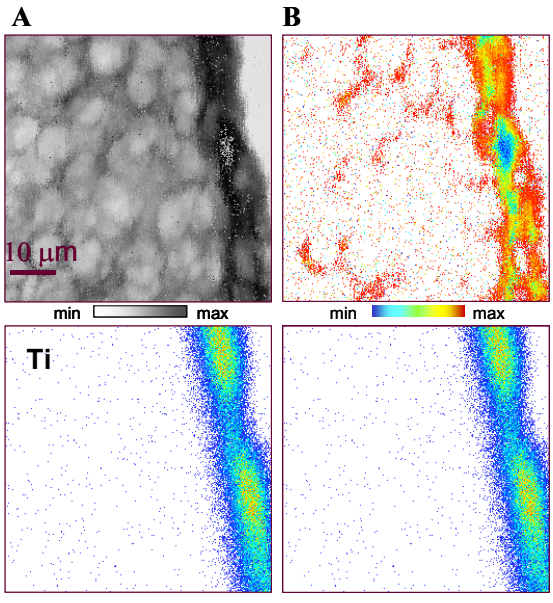

Fig.1. High-resolution transmission image (A) of pig skin evidencing the horny layer (B) and the Ti distribution map below. The overlapping of images (bottom right) show that Ti does not surpass the horny layer of skin.

High-resolution STIM images can be used to identify different layers of skin, as cell resolution is achieved. As shown in Fig. 1, different nuclear microscopy images obtained (e.g., X-rays, backscattered particles and transmitted particles) provide information related with the structure of the tissue and elemental distribution.

The profiles of P, Cl, and K enable to distinguish major skin strata, which are better evidenced by high-resolution transmission images of skin sections. There are no evidence of penetration of Ti below the horny layer of the skin (Fig.2).

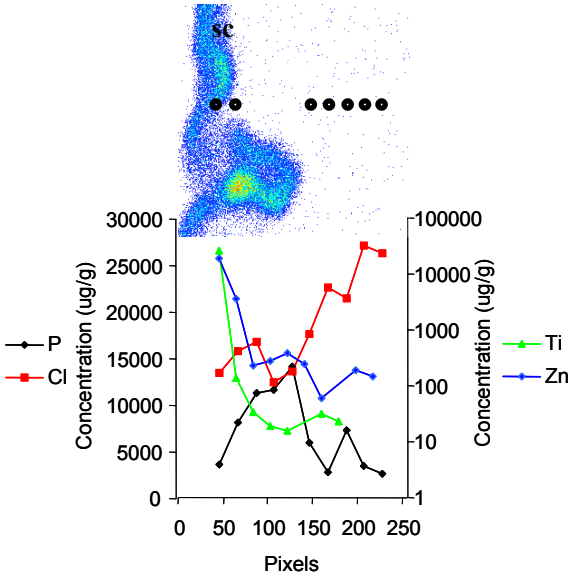

Fig. 2. High-resolution transmission image of a human skin section exposed to a product containing Ti and Zn oxides. Ti distribution image (colour) is overimposed. The horny layer (sc), epidermis (epi) and dermis (d) regions are evidenced. Below the elemental profiles of P, Cl, Ti and Zn across skin are plotted.

Published, accepted or in press work

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[1] Clínica de Dermatologia, FM-UL, Portugal
[2] Universidade do Minho, Portugal

Redox Status in Human Pathologies

T. Pinheiro, A. Veríssimo, R. Silva, J.N. Silva, P. Filipe, R. Fleming[1], A.B. Almeida[2], P. Monteiro[2], A. Barreiros[3], M.C. Santos[4], A.M. Viegas-Crespo[5], M. L. Mathias[5], P. Napoleão[5], C. Marques[5], I. Batista[5]

Objectives

The human physiological aspects such as those related to human diseases highly prevalent in the western population have been the prime areas of research. Other aspects related to the metabolism of essential trace elements, such as those occurring in human haemochromatosis are also being considered. The common objective of these studies is to seek for alternative indicators of tissue response, whether this response is caused by external or systemic factors.

Results

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Alterations in systemic indicators of oxidative stress such as elemental concentrations, antioxidant enzyme activities, protein and lipid oxidation and their relationship with the allelic profile of ApoE protein of LDL (plasma lipoprotein) are being studied in patients with atherosclerosis. Decreased K, Ca and Fe contents were observed in blood of stable patients relative to controls (Fig.1). In these patients a high incidence of ε4 allele was found which is associated to elevated LDL cholesterol levels.

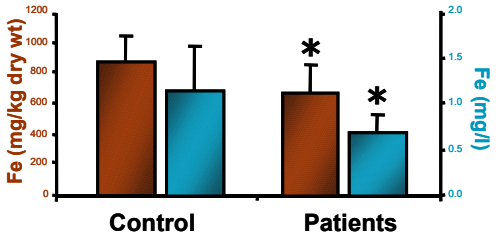

Fig. 1. Blood cells (red) and plasma (blue) Fe concentrations in atherosclerosis patients and healthy individuals. Significant differences to controls are indicated (*).

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Chronic Obstructive Pulmonary Disease (COPD) is a highly prevalent disease (affecting ca. 5% of the Portuguese population) associated with airway inflammation. The relationship between functional parameters, and indicators of redox balance and inflammation are being studied. In stable patients with COPD, plasma contents of protein carbonyls, total thiols and blood anti-oxidant enzyme activities were increased while plasma Se concentrations decreased being even more reduced in severe COPD patients with low levels of arterial oxygen pressure (Fig. 2). These changes could not be associated to the smoking history and/or habits of the patients.

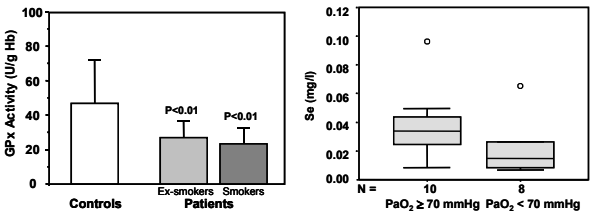

Fig. 2. Systemic variations of glutathione peroxidase activity and Se concentration in classes established according to smoking habits and blood gases respectively for the study groups.

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The deposition of Fe in skin of patients with haemochromatosis, as an accessible organ affected by this disease can be used to monitor the body Fe status along the therapeutic programme. So far nine patients at an initial stage of the disease were enrolled in the study. The epidermal Fe concentration is correlated with plasma Fe and ferritin contents. Patients will be followed before, after and six month after stopping the initial phase of the phlebotomy programme in order to establish the quality of skin to monitor haemochromatosis.

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4. P.A. Lopes, et al., Preliminary report on redox balance and blood elemental levels in atherosclerosis: relationship with Apo E polymorphism, *Proc. XIV Congresso Nacional de Bioquímica*, Vilamoura, Portugal, 2-4 December, 2004.

[1] Clínica de Hemoterapia, HSM, Portugal
[2] Clínica de Pneumologia, FM-UL, Portugal
[3] INETI, Lisboa, Portugal
[4] Centro de Química e Bioquímica, FC-UL, Portugal
[5] Centro de Biologia Ambiental, FC-UL, Portugal