Infrared Spectroscopy Determining the Biochemical Changes in Premalignant Skin Lesions Submitted to Photodynamic Therapy

Cassio Aparecido Lima[a], Luciana Correa[b], Denise Maria Zezell[a]

[a] University of São Paulo, Nuclear and Energy Institute Research, Av. Prof. Lineu Prestes 2242, 05508-000, Sao Paulo-SP, Brazil
[b]University of São Paulo, School of Dentistry, Av. Prof. Lineu Prestes 2227, 05508-000, Sao Paulo-SP, Brazil

The routine molecular techniques used to study cancer cells are time-consuming and require multiple assays/staining to study tissue-samples. On the other hand, FTIR provides a rapid and label-free analytical tool, which is able to study the molecular interactions occurring between biomolecules and not only on a single one. The present study aims to demonstrate the usefulness of FTIR spectroscopy to detect the biochemical changes induced by photodynamic therapy in dysplastic cutaneous lesions, as well as to interrogate the therapeutic results obtained by the treatment using 5-aminolevulinic acid (ALA) and its methyl ester (MAL) as photosensitizer prodrugs.

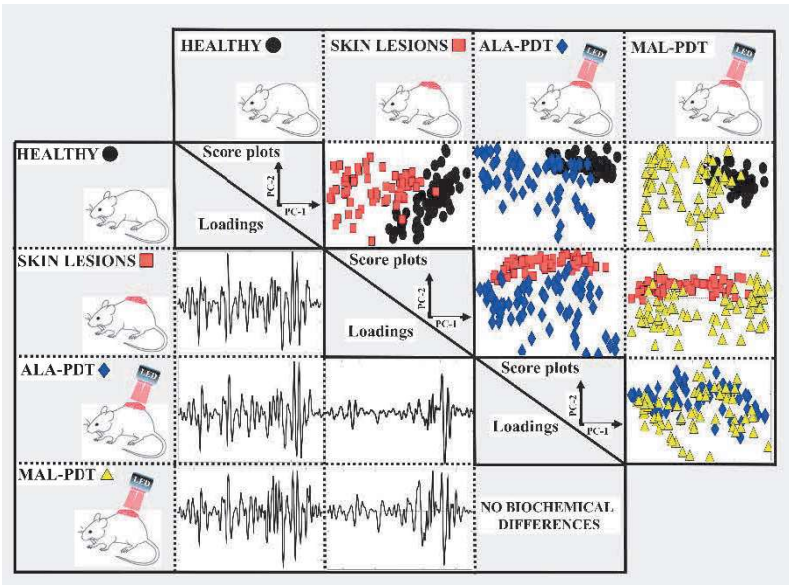

Figure 1. Results obtained for pairwise comparisons performed between untreated and PDT-treated cutaneous lesions against healthy skin. The score plots obtained for the first two principal components for each pairwise comparison are depicted above the main diagonal of the data matrix and the loading plots associated to the PC that better discriminated the data are depicted below the main diagonal.

Swiss mice underwent to a chemical carcinogenesis protocol aiming to induce the cutaneous lesions and were submitted to a single session of ALA/MAL-PDT. Spectra were collected from tissue sections of 5 μm thickness in the range of 4000 – 400 cm^{-1} using a Fourier transform infrared spectrometer on Attenuated Total Reflectance (ATR) sampling mode. The spectra second derivatives were submitted to the Principal Components Analysis to assess the biochemical changes promoted by the treatment. Overexpression of proteins (Amide I and II vibrations) was evidenced comparing non-treated and post-PDT tissues to healthy skin, as well as a decrease in the band intensity of collagen fibres and glycogen content. PDT-treated tissue exhibited similar histological characteristics to untreated lesions, indicating the presence of remaining lesions after treatment. FTIR spectroscopy revealed an increase in the amount of α-helix and β-sheet secondary structure of proteins on the post-PDT lesions, indicating that treated and non-treated tissue are biochemically different despite their morphological similarities.