

FTIR for the study of the biochemical changes promoted by PDT mediated by 5-ALA and its methyl-ester in neoplastic skin lesions

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The present study aims to demonstrate the ability of Fourier Transform Infrared (FTIR) spectroscopy on Attenuated total reflection (ATR) sampling mode associated with principal component analysis (PCA) to evaluate the biochemical changes caused by photodynamic therapy (PDT) in skin neoplastic tissue.

Cutaneous neoplastic lesions were chemically-induced in Swiss mice and submitted to a single session of PDT using 5-aminolevulinic acid (ALA) and its methyl ester (MAL) as photosensitizers. Tissue sections with 5 μm thickness were obtained from formalin-fixed paraffin-embedded (FFPE) and processed prior to the histopathological analysis and spectroscopic measurements. Spectra were collected in mid-infrared region using a Fourier transform infrared spectrometer on (ATR) sampling mode. PCA technique was applied on preprocessed second derivatives spectra and the biochemical changes were assessed using PCA-loadings and score plots.

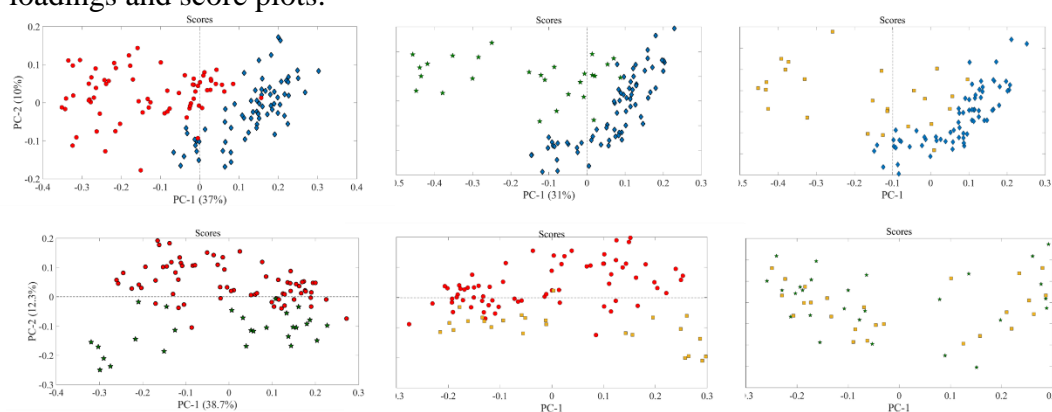


Fig. 1. Score plots obtained for the pairwise comparison between healthy skin (blue diamonds), non-treated neoplastic tissue (red circles), neoplastic skin treated with PDT using ALA (green stars) and post-PDT neoplastic tissue mediated by MAL (yellow squares).

We observed that PC-1 was the best principal component to discriminate healthy skin from the other groups. PC-1 loadings showed an increase in the vibrational modes related to Amide I and II in the non-treated and post-PDT neoplastic tissue compared with healthy skin, as well as a decrease in collagen fibers (1204, 1236, 1282 and 1338 cm^{-1}) and glycogen (1028, 1082 and 1151 cm^{-1}) content. Comparison between neoplastic tissue and post-PDT lesions showed that PC-2 was the best principal component to discriminate the groups and revealed a shift in the peak position, as well as a decrease in the β -sheet secondary structure of proteins (1624 cm^{-1}). Biochemical changes were not observed in the score plots and PC-loadings in the pairwise comparison of post-PDT neoplastic skin using ALA and MAL, which is in agreement with the morphological features observed in the histopathological analysis. Thus, we conclude that FTIR is a method that can be used for early diagnosis of premalignant skin lesions, as well as to evaluate the response to photodynamic treatment.

Acknowledgements

This work was supported by grants: FAPESP/CEPID (05/51689-2); CNPq/INCT (573.916/2008-0) and CAPES/Procad (8881.068505/2014-01).