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Discrimination of healthy skin and cutaneous malignant lesions using FTIR spectra and their second derivatives: A comparative study

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Abstract: PC-LDA statistical method was used to assess the accuracy of discrimination obtained by raw FTIR spectra and their second derivatives to discriminate cutaneous neoplastic tissue and healthy skin.

1. Introduction

Vibrational spectroscopic methods, including Raman and Infrared (IR) spectroscopy, have been fully succeeded analyzing biological samples. Spectroscopic research groups worldwide have used different approaches to assess the biochemical information retained by FTIR spectra using the raw spectra, as well as their second derivatives associated to multivariate statistical methods [1-4]. Thus, the present work aims to evaluate the accuracy of discrimination obtained by PC-LDA method applied to raw FTIR spectra and their second derivatives.

2. Material and Methods

Chemical carcinogenesis and sample preparation

Neoplastic lesions were induced on Swiss mice using a well-established multi-stage chemical-carcinogenesis protocol [5]. Excisional biopsies were obtained and the tissue were formalin-fixed and paraffin-embedded (FFPE). Sample sections with 5 µm thickness were obtained and placed in MirrIR low-E-coated slides (Kevley Technologies, Chesterland, OH, USA) and submitted to dewaxing protocol before spectroscopic analysis.

FT-IR Spectroscopy

Conventional macro-ATR-FTIR measurements were collected in the range of 4000–400 cm^{-1} using a Thermo Nicolet 6700 FTIR system (Thermo Scientific, Waltham, MA, USA) on Attenuated total reflection setup (ATR). The data non-submitted to derivative operation were only vector-normalized, whereas the derivative group were submitted to second derivative and vector-normalization. For signal-smoothing, spectra were submitted to Savitzky–Golay filter with a polynomial of second order in an eleven points window. Principal Component Analysis (PCA) was performed for both cases and the first principal component was used as input to Linear Discriminant Analysis (LDA) aiming to assess the accuracy of discrimination of experimental groups.

3. Results and Discussion

Figure 1.A and B show the averaged FTIR spectra and their second derivatives obtained for healthy and neoplastic tissue, respectively. The vibrational modes peaking at wavenumber range from 900-1800 cm^{-1} are associated to important cell content, therefore this region has been widely used to describe the biochemical features of sample-tissue.

Comparing the vibrational modes identified in the raw spectra and their second derivatives we observed that all bands associated with glycogen (1028, 1082 and 1151 cm^{-1}) [7], as well as nucleic acids (1082 and 1236 cm^{-1}) [7] were identified in both cases. Bands peaking at 1236 and 1338 cm^{-1}, which are related to collagen fibers, were also observed in both cases. Nevertheless, the collagen bands at 1204 and 1282 cm^{-1} [7] were identified only in the derivative spectra. The wavenumbers peaking at 1500-1700 cm^{-1} comprise the Amide I and II region, which were identified in both spectra and second derivative. However, only the second derivative enabled the identification of the sub-bands overlapped in this region (1624 and 1650 cm^{-1}) [7], which are associated to the secondary structure of proteins.

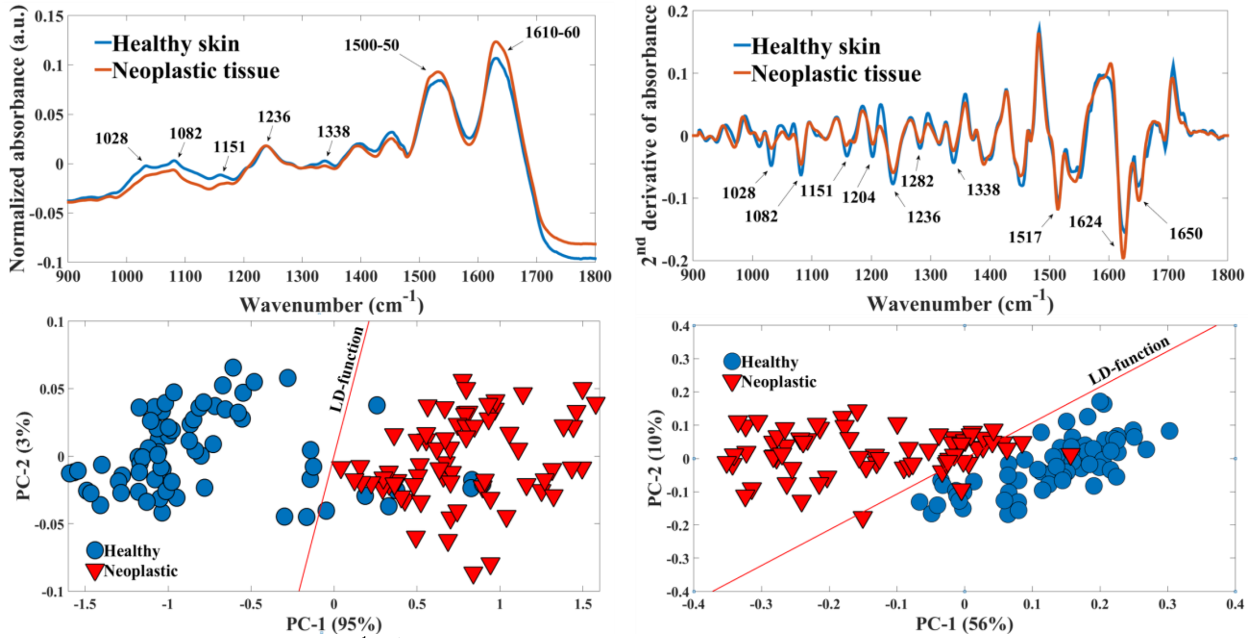

Fig. 1: Fingerprint region (900-1800 cm^{-1}) of averaged vector-normalized FTIR spectra (A) and their second derivatives (B). Healthy skin is depicted in blue line and neoplastic tissue in red. Figures C and D display the PCA score plots obtained for pairwise comparison between the groups. Blue circles represent healthy skin and red circles represent neoplastic tissue.

Spectra were submitted to PCA technique in order to assess the data discrimination obtained by the score plots of PC-1 *vs.* PC-2 using the spectra (Figure 1.C) and derivative spectra (Figure 1.D). For both cases the first principal component was the best PC to discriminate healthy and neoplastic skin. Finally, the PC-LDA method obtained an overall accuracy of 89% to discriminate healthy skin from neoplastic tissue using the spectra, whereas the method obtained 89% using the second derivatives as input data. The satisfactory specificity obtained by FTIR spectra indicates that the method is a good identifier for neoplastic spectra. On the other hand, the second derivative is a good method to detect healthy spectra;

4. Conclusions

In this study, PC-LDA statistical method was used to assess the discrimination between cutaneous neoplastic tissue and healthy skin using FTIR spectra and their second derivatives as input data. FTIR spectra presented as a good identifier of neoplastic spectra and the second derivatives for healthy spectra. However, the second derivative method was the only one able to identify the overlapped sub-bands associated to the secondary structure of proteins in the Amide I region (α-helix at 1650 cm^{-1} and β-sheet at 1624 cm^{-1}), as well as the vibrational modes associated to the collagen bands at 1204 and 1282 cm^{-1}. Thus, both methods may be used for classification purposes. On the other hand, for exploratory analysis, second derivative is indicated due to the higher available information.

5. References

[1] Silveira FL, Pacheco MTT, Bodanese B, Pasqualucci C, Zangaro R, Silveira L, "Discrimination of non-melanoma skin lesions from non-tumor human skin tissues in vivo using Raman spectroscopy and multivariate statistics", Lasers Surg Med **47**, 6–16 (2015).

[2] Vyumvuhore R, Tfayli A, Manfait M, Baillet-Guffroy A., "Vibrational spectroscopy coupled to classical least square analysis a new approach for determination of skin moisturizing agents' mechanisms", Ski Res Technol **4**, 282–292 (2014).

[3] Cassio A. Lima, Viviane P. Goulart, Luciana Correa, Thiago M. Pereira, Denise M. Zezell, "ATR-FTIR Spectroscopy for the assessment of biochemical changes in skin due to cutaneous squamous cell carcinoma", Int J Mol Sci **16**, 6621–6630 (2015).

[4] Cassio A. Lima, Viviane P. Goulart, Luciana Correa, Denise M. Zezell, "Using Fourier Infrared spectroscopy to evaluate biological effects induced by photodynamic therapy", Lasers Surg Med, *available online* (2016).

[5] E. Abel, J. Angel Angel J, Kiguchi K, DiGiovanni J. "Multi-stage chemical carcinogenesis in mouse skin: Fundamentals and applications", Nat Protoc **9**, 1350–1362 (2009).

[7] Z. Movasaghi, S. Rehman, IU. Rehman, "Fourier transform infrared (FTIR) spectroscopy of biological tissues", Appl Spectrosc Rev **43**, 134–179 (2008).

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