Journal of Photoscience (2002), Vol. 9(2), pp. 457–459

A Method for Observation of Benign, Premalignant and Malignant Changes in Clinical Skin Tissue Samples via FT-IR Microspectroscopy

Natalja Skrebova[1*], Katsuo Aizawa[2], Yukihiro Ozaki[3], Seiji Arase[1]

[1]Department of Dermatology, Tokushima University, Tokushima 770-8503, Japan

[2]Department of Physiology, Tokyo Medical University, Tokyo 160-8402, Japan

[3]Department of Chemistry, Kwansei-Gakuin University, Sanda 669-1337, Japan

Sunlight causes various types of adverse skin changes on the sun-exposed areas of the skin, in which the most hazardous one is the induction of malignant skin tumours. FT-IR spectra were obtained from specimens excised from normal skin, BCCs, SCCs, MMs, nevi, lesions of solar keratosis and Bowen's disease. Tissue samples from freshly frozen specimens were cut into 2 sections in strictly sequential order to be stained with H & E for histopathological analysis, and then to be air-dried on CaF_2 slide glasses for further spectral data acquisition from defined area of interest. Intra- and inter-sample variations were estimated within grouped lesion categories according to each skin component. Mean spectra for each type of tissue pathology in the 800-1800 cm^{-1} region was interpreted using the classical group frequency approach that showed the most visible differences in spectra of benign, premalignant and malignant changes directly related to protein conformation and nucleic acid bases. The relative intensity of the nucleic acid peak was increased with progression to malignancy. In addition, PCA was able to evaluate and maximise the differences in the spectra by reducing the number of variables characterizing each patient and pathology category. This type of approach to non-destructively estimate the complexity of IR-spectra of inhomogeneous samples such as skin demonstrates the advantage of FT-IR microspectroscopy to be able to observe diseased states (benign, premalignant, malignant) and distinguish them from normal against a huge background of inter- and intra-subject variability.

Key words: Infrared microscopy; Fourier transform infrared spectroscopy; skin cancer; diagnosis

INTRODUCTION

Recently IR-microspectroscopy has been evaluated as a

* To whom correspondence should be addressed.

E-mail: n_skrebova@hotmail.com

Abbreviations: IR, infrared; *FT,* Fourier transform; *BCC,* basal cell carcinoma; *SCC,* squamous cell carcinoma; *MM,* malignant melanoma; *H & E,* hematoxylin and eosin; *CaF_2,* calcium fluoride; *PCA,* principal component analysis.

complementary technique for *in vitro* cancer diagnostics of various organs, including skin [1]. One of the great advantages of this method is that it provides an enormous amount of information about the structure of proteins, nucleic acids, lipids and carbohydrates contained in infrared spectra, thus suggesting IR-spectroscopy as a possible tool to detect and monitor normal and diseased processes in cells and tissues [2].

In the present *in vitro* study, we examined spectra of benign (nevi), premalignant (Bowen's disease, solar keratosis) and malignant (BCC, SCC, MM) lesions, in comparison to histopathologically normal skin, in order to observe processes from benign to malignant transformation via FT-IR microspectrometry.

MATERIALS AND METHODS

Sample collection

Fresh specimens of BCCs, SCCs, MMs, nevi, solar keratosis and Bowen's disease were collected during routine surgical procedures. Each tissue sample was cut into pieces – to be freshly frozen and stored in the deep freezer for future spectroscopic experiments, and to be stained with H & E for histopathological diagnosis.

Sample preparation

After a pathological diagnosis had been established, in which histological features or tumor growth patterns were described for each lesion, tissue samples from freshly frozen specimens were cut into 7 μm sections in strictly sequential order to be stained with H & E in order to orient collection of spectra from the unstained section, and then to be air-dried on CaF_2 slide glasses for further spectral data acquisition from defined areas of interest.

Infrared microspectroscopy

An FT-IR microspectrometer (IR-MAU200, JEOL Co, Tokyo, Japan) was used to obtain spectra with a resolution of 4 cm^{-1} over a spectral range of 800-3500 cm^{-1}, using a knife edge aperture reduced to 25 × 25 μm.

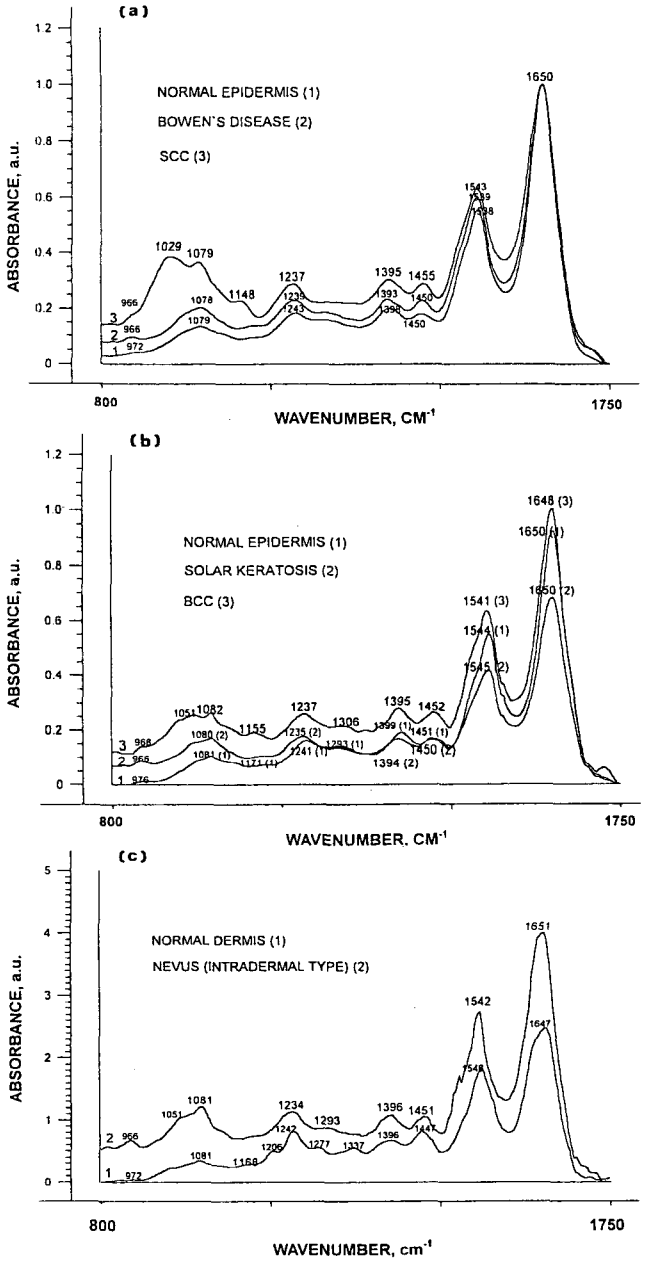
Data treatment

The techniques applied to the spectral data include: 1) baseline correction; 2) smoothing and normalization (if desired); 3) spectral bands assignment using the classical group frequency approach [1, 2]; 4) absolute peak intensity or peak intensity ratio measurements. In addition a multivariate technique, PCA, was performed on the tissue spectra to estimate intra- and inter-sample variation within grouped lesion categories according to each skin component (epidermis, dermis, the lesion).

RESULTS AND DISCUSSION

Various post processing techniques that have been applied to the spectral results revealed specific features of the sample: absorption between 1000 and 1150 cm^{-1} seems to correlate with a variation of the amide $I_{1600\text{-}1700\ cm^{-1}}$/amide $II_{1480\text{-}1575\ cm^{-1}}$ intensity ratio; the spectral features due to DNA and amide III (965 cm^{-1}, 1071 cm^{-1}, 1084 cm^{-1}, 1095 cm^{-1}, 1245 cm^{-1}) have been modified and enhanced with progression to malignancy. Representative spectra in the 800-1800 cm^{-1} region for each type of tissue pathology of epidermis or dermis showed the most visible differences in spectra of benign, premalignant and malignant changes directly related to protein

conformation and nucleic acid bases, compared to spectra from equivalent normal skin components (Figs. 1a-c).



Figs. 1a-c. Representative IR-spectra in the 800-1750 cm^{-1} region of epidermal premalignant (Bowen's disease, solar keratosis) and malignant (SCC, BCC) tumours, benign pigmented lesion (nevi) in comparison with normal epidermal and dermal skin components.

PCA was able to evaluate and maximise the differences in the spectra by reducing the number of recognizable variables characterizing each patient and pathology category (Fig. 2).

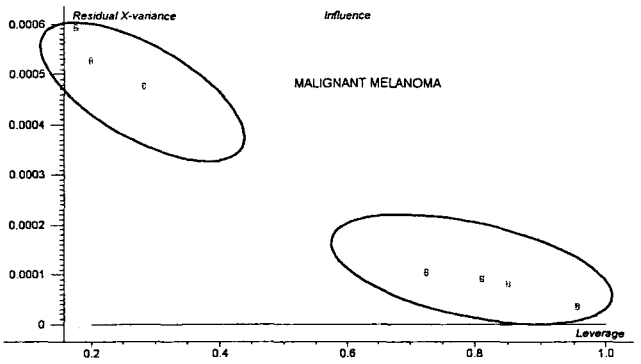


Fig. 2. An example of partitioning together spectra of MM obtained in epidermis and dermis (intra-sample variability) based on measurement of its variance.

This type of approach to non-destructively estimate the complexity of IR-spectra of nonhomogeneous samples such as skin demonstrates the advantage of FT-IR microspectroscopy enable observation of diseased states, like benign, premalignant and malignant, and distinguish them from normal against a huge background of inter- and intra-subject variability.

REFERENCES

1. Dukor, R.K. (2002) Vibrational spectroscopy in the detection of cancer. *In: Handbook of Vibrational Spectroscopy (Vol. 5)*. Ed. by Chalmers J, Griffiths PR, John Wiley & Sons Ltd, pp.3335-61.
2. Diem, M. et al. (1999). Infrared spectroscopy of cells and tissues: shining light onto a novel subject. *Appl. Spectrosc.* 53, 148-61A.