

A Study of Human Oral Cavity Cancer Tissues Using Synchrotron-Based Infrared Microspectroscopy

Pei-Yu Huang (黃佩瑜)¹, Ching-Iue Chen (陳慶曰)¹,
Yao-Chang Lee (李耀昌)¹, and Da-Bin Sheng (謝達斌)²

¹National Synchrotron Radiation Research Center, Hsinchu, Taiwan

²Institute of Oral Medicine, Medical Colleges, National Cheng Kung University, Tainan, Taiwan

Using infrared microspectroscopy (IMS) can be considered as a fast and non-invasive tool for investigating molecular and supermolecular structure of cancer cells and tissues with no need of laborious and invasive sampling procedures. In this study, we reported chemical images based on characteristic infrared absorption of biomolecules for differentiating normal and malignant human oral tissue from the same patient. The infrared spectra of malignant tissue revealed that (i) the absorbance for amide I and amide III band at 1642 cm^{-1} and 1233 cm^{-1} , respectively, in the malignant part of tissue is much stronger than that of the normal, (ii) the characteristic band for phospholipids at 1740 cm^{-1} ($\nu_{\text{as}}\text{C=O}$) was only found within epidermis of normal tissue, (iii) and absorbance of lipid at $2880\text{--}2990\text{ cm}^{-1}$ is lower in intensity than that of normal tissue.

Oral tissue samples were frozen on excision and cryomicrotomed to a nominal thickness of $5\text{ }\mu\text{m}$. Sections were then mounted onto low-e slide and air dried for FT-IR microanalysis. The adjacent sections for histopathological examination were mounted on a microscopic glass slide and stained with hematoxylin and eosin (H&E). The samples of tissue were measured by conventional FTIR microspectroscopy using the aperture of $30\times 30\text{ }\mu\text{m}^2$ and step size was $30\text{ }\mu\text{m}$ for tissue section. Spectra were coadded for 64 scans for tissue sections.

Results and Discussion

General biochemical mappings regarding the specific components of protein, lipid and DNA were obtained using chemical imaging method. The white light image of human oral normal (point A is epidermis tissue) and malignant (point B is cancerous region and point C is precancer region) tissues of the same patient shown as Fig.2a. The A, B and C with marked spots of single measurements of FTIR spectra in the region from 990 to 3500 cm^{-1} . The data shown in Fig. 2b, the band of 1080 cm^{-1} is due to vibration of the phosphodiester groups in DNA that can be used to identify the content of cellular nucleus. The infrared spectra revealed that the absorption of the band at both center of 1247 cm^{-1} and 1080 cm^{-1} for malignant tissue is stronger than that of normal tissue, and we assigned them to an asymmetric and symmetric stretching vibration of PO_2^- of residual of DNA/ RNA. Based on the behavior of intensity of PO_2^- , we proposed that the malignant part of tissue was with higher cell population than that of normal part. The absorption for lipid at 1740 cm^{-1} ($\nu_{\text{as}}\text{C=O}$) was found at normal epidermis tissues but absent of malignant tissue. The malignant tissue of lipid absorption was lower in intensity than that the normal. Additionally, in those regions, there

are N–H stretching vibrations as well as bands belonging to amide A at 3300 cm^{-1} and amide B at 3075 cm^{-1} in proteins and nucleic acids. In the study of pathological states, potential spectroscopic markers of some neoplasia have already been identified by determining the values of the absorbance ratios $1030/1080$, $1080/1247$, $1445/1247$ and $2960/2853\text{ cm}^{-1}$ for precancer and cancer of human oral cavity cancer tissue shown as Fig. 2.

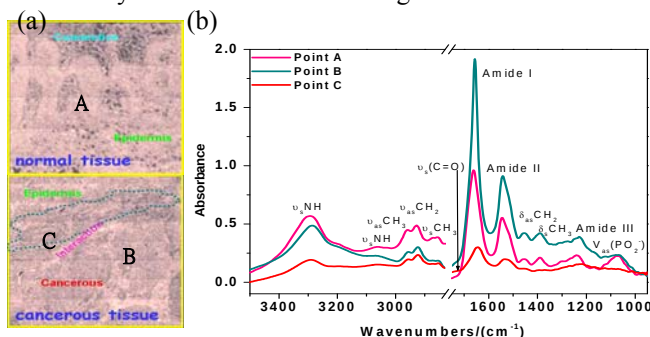


Figure 1. (a) Visible images of human oral normal and malignant tissues of the same patient, (b) FTIR absorbance spectra of human oral normal, precancer and cancer tissues for the spectra region between 990 and 3500 cm^{-1} .

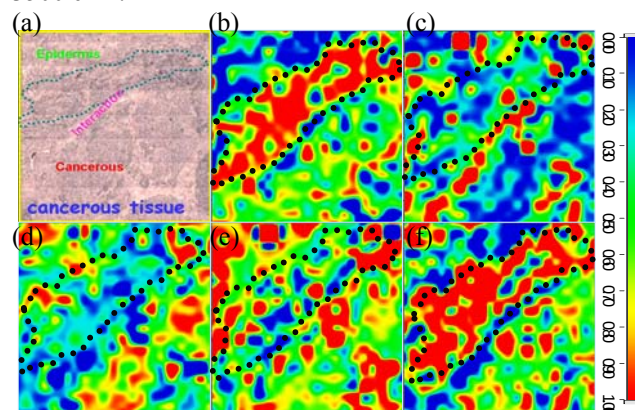


Figure 3. (a) Visible images of human oral malignant tissues (b) $1030/1080$, (c) $1080/1247$, (d) $1325/1080$ and (e) $2960/2853\text{ cm}^{-1}$ absorbance band ratios map for cancers of human oral cavity cancer.

Conclusion

Significant differences between normal and malignant oral cavity tissues were seen in FT-IR spectra in this study. In our case, we can identify some spectral ‘biomarkers’ to distinguish among precancer and cancer conditions. In addition, the absorbance ratio of $1325\text{ cm}^{-1}/1080\text{ cm}^{-1}$ for the malignant tissue is higher than that of premalignant region. occurs, as verified in the sarcomatoid tumor.