diagnosis, be it false positive or false negative, can have serious consequences. A false positive leads to unnecessary anxiety and diagnostic procedure, whilst a false negative may delay the discovery of premalignant lesion or a carcinoma until a later stage. The screening procedure involves a microscopic search by eye, for the small percentage of abnormal cells from hundreds of thousands of normal cells on a smear, which is fatiguing, time consuming and reliant on human judgment. In order to improve the inconsistencies occurring from Pap smear misdiagnosis, a more reliable means of cancer screening is desirable. To solve these problems, we need to explore new method for diagnosis and prognosis of precancer and cancer. Fourier transform infrared spectroscopy (FTIR) could be one of the methods, which provides information at the molecular level that might help to find explanations and solutions for the early detection and progression of cancer.

Fourier transform infrared spectroscopy (FTIR) records the interaction of IR radiation with biological samples, measuring the frequencies at which the sample absorbs the radiation and intensities of the radiation. Determining these frequencies and intensities enables identification and interpretation of the sample's molecular composition. Cancer development is preceded or accompanied by molecular changes and intermolecular interactions in the cells. IR spectroscopy could reveal such changes, which are not yet evident to the physician and hence form the basis for the sensitive diagnosis. Although FTIR has been known for fifty years in chemical and pharmacological industries, it was hardly used for human biological applications, due to complexity of the biological material. Even then, recent studies have firmly reported that FTIR technique is becoming a uniquely powerful tool for determining biochemical composition within a biological system and for monitoring the physical state of the biochemical compounds[5]. FTIR spectroscopy can detect conformational changes in cellular composition that reflect the onset of cancer[6] and intermolecular interactions in the cell[7].

ADVANTAGES OF THE FTIR SPECTROSCOPY

- FTIR technology is sensitive, and requires very small amount of samples (5-10 μl) for analysis. Samples in a variety of forms and physical states can be studied.
- None of the microscopic techniques available today provide detailed distributions of proteins, nucleic acids, carbohydrates and lipids inside a living cell. Infrared (vibrational) spectroscopy is currently evaluated in order to obtain such information. Less power source is required to record high quality IR spectra, thus preserving tissue viability.
- As the technique is based on visualization, FTIR spectroscopic images can easily be compared and correlated with standard histological results. Acquisition of IR spectra with high spatial and spectral resolution allows the visualization of the distribution of intrinsic biochemical components such as proteins, nucleic acids, carbohydrates and lipids.
- The technique rapidly collects thousands of spectra, each reflecting a myriad of biochemical and structural aspects of the sample, many statistical and multivariate approaches can be utilized to analyze and interpret the data[8 9].
  Spectroscopic methods require specific markers or stain to detect cancer related to changes in cells and tissues. FTIR spectroscopy does not require such markers, which are utilized to detect precancer and cancer before it is visible morphologically.
- IR spectroscopic studies on biological tissues and fluids were mostly performed with the aim of developing diagnostic techniques. If the characteristic spectrum of cancer or normal tissue component is known, it may be possible to compare the spectra in each cluster to these reference spectra, allowing an objective biochemical / histological description of tissues. Development of such a methodology would firmly establish infrared microscopy as a powerful diagnostic tool to support the result of histopathological determination of cancer[10].

THE MECHANISM OF SAMPLE ANALYSIS

The IR energy is emitting from the black body source and passes through an aperture, which controls the amount of energy applied to the sample. The beam enters the interferometer where the "spectral encoding" takes place. The resulting interferogram signal then exits the interferometer. Then the beam enters the sample compartment where it is transmitted through or reflect off the surface of the sample, depending on the type of analysis being performed. This is where specific frequencies of energy, which are uniquely characteristic of the sample, are absorbed. Finally, the beam passes to the detector to measure the special interferogram signal. The measured signal is digitized and sent to the computer where Fourier transform takes place. The final infrared spectrum is then used for interpretation and further analysis.

FTIR SPECTROSCOPIC SCREENING OF CERVICAL PRECANCER AND CANCER

FTIR analysis on human cells and tissues was successfully initiated in 1980's by few research groups,

in particular Prof. Wong's group from Canada[7]. Later ,due to its tremendous potential, simple protocol and easy operation, it was adopted by several research groups on various disciplines throughout the world. Among the various disciplines chosen and worked, studies on cervical cells and tissues attained great impact and significant progress towards the clinical evaluation. Several studies consisting of single cervical cell, groups of cells, tissues, and tissue sections have been carried out and they were described in the present article.

Wong et al[7] reported IR spectra of normal women's cervix differed from those obtained from patients with either dysplasia or cancer. In malignant cases, they noted significant changes in intensity of glycogen bands at 1025 and 1047 cm^{-1}, bands at 1082 and 1244 cm^{-1}, C-O stretching band at 1155 cm^{-1} and band at 1303 cm^{-1}. Significant frequency shift at 1082, 1155 and 1244 cm^{-1} and an additional band appeared at 970 cm^{-1}. In malignant cervical tissue there were extensive changes in the degree of hydrogen bonding of phosphodiester groups of nucleic acids and C-OH groups of proteins as well as changes in methylene chains of lipids. The IR spectra of dysplasia showed same changes with cancer samples except that the magnitude of change was lesser and no shift of band at 1082 cm^{-1}. Pressure tuning is another method of screening in which FTIR spectroscopy combined with pressure effects on spectral features, such as band frequencies, intensities, splitting, resonance and so forth. Unlike temperature, a change in pressure affects only the space available to the molecules without changing their kinetic energy. Consequently, the degree of intermolecular interactions can be directly varied with pressure and thus new knowledge on intermolecular interactions and structural properties can be obtained. Using this method Wong et al[11] extracted structural differences in normal connective tissue, normal epithelial cells and malignant tissue from 7 patients in the regions of symmetric and asymmetric phosphodiester groups, C-O stretching mode, C-H bending mode and the amide I. The IR spectrum of normal connective tissue of cervix in the region 950-1100 cm^{-1} is similar to that of malignant cells and tissue. However, spectral features of normal connective tissue were differentiated unambiguously from the malignant tissue and normal epithelial tissue in the region 1200-1500 cm^{-1}. Therefore if only the region 950-1100 cm^{-1} examined, the normal connective tissue can be misinterpreted as malignant tissue. This study highlights various regions of the spectrum to be identified and analyzed carefully in order to differentiate the abnormal from the normal.

Fung et al[12] compared exfoliated cervical cells from 301 patients between the FTIR spectroscopy results and cytology cum colposcopy guided biopsy results. They provided distinct definitions of false negative / false positive FTIR, cytology and histology. Result of 301 patients showed 196 positive and 105 negative cytologies. The sensitivity, specificity, false positive and false negative rates were 86.6%, 90.5%, 13.4%, and 9.5% respectively in cytological examination. However, FTIR results versus histology showed 215 positive and 86 negative. The false positive and false negative rates were 1.4 and 1.2% respectively. In the 12 cancer cases tested, there was no false positive FTIR result, but 3 false negative Pap test results were obtained. The positive and negative predictive values for FTIR were 99.5% and 96.5 % respectively while the Pap test values were 95.6% and 72.3% respectively. Based on these findings, they concluded that FTIR has a better false negative and false negative predictive value compared to Pap test. Chiriboga et al[14] studied infrared spectral pattern of formalin fixed, paraffin embedded tissue sections of normal, dysplastic and malignant cervical samples. Spectral variations found in paraffin embedded sections were far less than those observed for single cell. Nevertheless, they found direct correspondence between spectral data obtained from tissue sections and individual exfoliated cells. They also found spectral properties of dysplastic samples were intermediate between spectral features of normal and malignant samples.

Fourier transform infrared microspectroscopy (FTIR-M) combined with Principal component analysis (PCA) in the study of exfoliated cervical cells from 272 patients showed two types of spectral pattern visually; type 1 and type 2. Type 1 represents profiles of normal cell characteristics; glycogen bands at 1025 and 1155 cm^{-1} and phosphate band at 1078 cm^{-1}. Type 2 represents characteristics of dysplastic and (or) malignant showing reduction in glycogen intensity and pronounced asymmetric phosphate groups. PCA score plot showed greater degree of separation between types 1 and 2[15]. Lowry[16] observed spectral features that have been attributed to the normal cervical samples and those related to abnormal cervical samples are relatively consistent across the total sample. This indicates that the spectral changes are not caused by a few bad cells in the normal as usually detected in the Pap smear screening but can be attributed to a chemical difference in the majority of the cells in the sample. Romeo et al[17] used IR spectral data of normal and dysplastic cervical smears as a databank to investigate the usefulness of artificial neural network (ANN) to differentiate dysplasia from normal. The results indicate that neural network coupled to infrared microspectroscopy could provide an alternative automated means of screening of cervical cancer. Shaw et al[1] obtained better classification accuracy for normal / CIN 3 than for normal / CIN 2 or normal / CIN 1 using linear discriminant analysis (LDA) and quadratic discriminant analysis (QDA). The success of the classification distinguishing normal specimen from the CIN 3 in particular provides strong

evidence that IR spectra carry genuine diagnostic information. Diem et al[18] introduced experimental and computational methods of infrared microspectroscopy and infrared spectral mapping on sections of various tissue types. They identified differences in total intensity, amide I / amide II ratio, protein / nucleic acid ratio glycogen and other factors in various tissue types. Diem et al[19] also reported spectral differences in individual normal and cancerous cells of cervix were mostly due to differences in DNA, RNA and phospholipids. Metabolically inactive cells show spectral differences in proteins only, whereas active cells show spectral differences of nucleic acids and phospholipids. Study by Romeo et al[20] in which definite spectral changes noted in carbohydrate region (1200-1000 cm^{-1}) can be attributed to accumulation of glycogen in the ectocervical cells throughout the menstrual cycle. They found that accumulation of glycogen in the ectocervical cells varies with different stages of the menstrual cycle, which is in turn controlled by the level of estrogen. Despite these cyclic differences, PCA was able to demonstrate that high-grade dysplasia could be separated from the normal. In another study Romeo et al[21] used PCA to separate potential confounding variables from normal and abnormal cervical smears. Tighter cluster was formed when the spectral region was reduced to 1096-1062 cm^{-1} although a better separation was not always achieved by reducing the variables. Wong et al[22] described the detailed accounts of confounding variables that lead to misinterpretation of cervical cells spectra. They have identified the IR spectra of polymorphs, cell degradation, endocervical columnar cells, metaplastic cells, cervical mucous, red blood cells and debris. Interpretation of cervical cells spectra must be carried out after subtraction of spectral influences from these factors, which is an essential step to prevent the occurrence of unacceptable false positive rates. In our lab, we identified and compared the IR spectral characteristics of exfoliated normal cervical cells, adenocarcinoma cells, adenocarcinoma tissues and adenocarcinoma cell line. Changes in spectral intensity ratios were recorded at 1025, 1080, 1155, and 1240 cm^{-1} in all malignant cases compared to normal. Spectral pattern observed in malignant cases was similar among the malignant cases, but varied from normal[23].

POSSIBLE APPLICATION

Atypical squamous cells of undetermined significance (ASCUS) shows cellular abnormalities that are marked than those attributable to reactive changes, but quantitatively or qualitatively short of a definitive diagnosis of squamous intraepithelial lesion. Nuclear enlargement may be two or three times that of normal Intermediate cells, and there may be minimal nuclear irregularity and mild hyperchromasia. These changes complicate to confirm diagnosis. FTIR technology may be used to investigate the frequency with which an ASCUS designation predicted neoplasia on biopsy. A study by Takezawa et al[24] in which all cervical smears read by the Department of Pathology, University of Florida between 1992 and 1995 were reviewed and classified as those within normal limits, those with benign cellular changes or those with epithelial cellular abnormality. 5.5-5.8% of the smears contained low-grade squamous intraepithelial lesions, and 3.0-5.5% contained high-grade intraepithelial lesion.

Glassy cell carcinoma of cervix is a rare and aggressive tumour, classified as a subtype of adeno squamous carcinoma. This carcinoma tends to occur in younger women than does squamous cell carcinoma. Its virulent biological behaviour is manifested by metastases occurring at an early stage and a high recurrence rate after conventional surgical or radiation therapy. It accounts for 1-2% of cervical cancer[25] and may be difficult to diagnose on conventional cytology, and in such cases FTIR may become a useful diagnostic tool.

CONCLUSIONS

According to the results discussed in the present review article, infrared spectroscopy is proving to be potentially valuable and versatile technique in the diagnosis of cervical cancer as it has been useful in the fields of chemistry, biology and biophysics. A sound understanding of the sampling methods and the spectroscopic features of the important building blocks of the cells and tissue coupled with appropriate multivariate analytical methods, such as PCA, LDA, and ANN allow impressive level of discrimination between normal and cancer samples. Various approaches taken on the analysis of cervical cells and tissues using the FTIR method attained a great success in screening and diagnosis. In the near future, we can expect to see FTIR spectroscopy as a reliable screening tool for cervical precancer and cancer.

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