Application of FT-IR Techniques to Non-invasive Clinical Diagnosis and its Future Role in Medicine

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We have developed recently the Fourier transform infrared spectral analysis system to measure constituents of human oral mucosa in vivo and clinically obtained serum for non-invasive diagnosis. The attenuated total reflectance (ATR) probe in rod shape was adopted for the measurements of both oral tissue and serum, with attaching the oral tissue in vivo and a small amount of serum solution on the probe. The results indicate that the change of in vivo oral mucosal infrared spectra correlated with that of blood triglyceride level, but the blood glucose level was hardly detected directly through mucosal tissues with this probe. When more sophisticated analytical methods are developed for multiple infrared data sets and for miniaturized instruments using optical fiber system, the application of Fourier transform infrared analytical technique would be more advanced in the field of non-invasive clinical diagnosis.

INTRODUCTION

Previously we measured the FITR spectra of rat artery, brain tissue, and human oral malignant tissues [1]. With these measurements, it has been proved that FTIR technique is useful to measure non-destructively and non-invasively the tissues from various origins. Several applications of FTIR techniques to human tissues have been carried out in order to compare malignant or abnormal state of tissues with the normal one [2]. However, the improvement of these techniques to apply on clinical diagnosis has not been advanced so far probably because of the lack of significant breakthroughs in the hardware and software developments especially in the medical field.

The infrared spectra of human tissues in high quality may provide significant chemical information about the constituents of tissues. If this advanced merit of the infrared analysis was correctly used in the clinical field, many applications would contribute to the non-invasive clinical diagnostic method and pilot study in the pathological diagnosis. In this article, I will summarize our data about the non-invasive and non-destructive measurements of human oral mucosa *in vivo* and serum using specially designed ATR probe with statistical analytical methods. Moreover, I will comment on the development of technology of infrared analyses in medical diagnostic field.

EXPERIMENTAL

Materials

Clinically obtained sera were provided by Central Clinical Laboratory, Fukushima Medical College. Fourier transform infrared spectra were measured with an ATR probe attached to the instrument (JIR-100, JEOL).

ATR measurements of human oral mucosa

Specially designed ZnSe ATR probe (rod-shape) was attached to FTIR machine, and a volunteer subject is asked to sit down with putting his(or her) oral mucosa (inside of lips) on the rod supporting with fingers. Measurement is done within 1 min. Spectra have been derived mainly from mucosa surface but not saliva.

Measurement of sera, clinical specimens

Sera were obtained from patients in clinically glucose-loaded test program, and 10 μL of serum was put on the ZnSe rod wrapping with aluminum foil stripe and sticking by clip.

RESULTS AND DISCUSSION

FTIR analysis of human oral mucosa in vivo

We can expect that the constituent of human oral mucosa is ready to be affected by the change of constituent of blood as the permeability of water or

other small molecules is high in the oral mucosa. I expected that the non-invasive monitoring of blood lipids or other constituents might be possible when I could measure the infrared spectrum of oral mucosa *in vivo*. For this purpose I have developed the specialized ATR probe system using ZnSe rod to measure human oral mucosa by attaching mouth mucosal surface on the

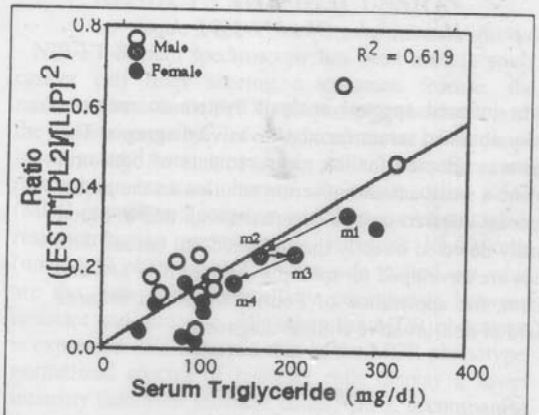

FIGURE 1. Correlation between serum triglyceride and infrared Lipid Factor (ratio) for volunteer subjects.

rod. With this system I could obtain the FTIR spectra of mixed mucosal lipids, glycosides and proteins *in vivo*. As this system employs ATR probe, materials present in the depth of several micrometer from the mucosal surface could only be measured. When the diurnal variation of the spectra of oral mucosa was measured, some sets of infrared bands were noticed to be well correlated with the intake of meals, and some bands, especially lipid-related bands, showed an increase 1 to 2 hours later after the lunch intake. This suggests that the lipid constituents in oral mucosa may be gradually affected by the blood lipid constituents.

Moreover, the correlation between blood triglyceride content and the FTIR spectrum of oral mucosa was measured, and a ratio among infrared bands originated from fatty acid methylene CH stretching band, fatty acid ester band and phosphate ester band (I named this ratio Lipid Factor) had a good correlation with the blood triglyceride level (Fig.1). This Lipid Factor did not correlate with the blood cholesterol level of the volunteer subjects. We are now improving the ATR probe to attach freely any point of oral mucosa.

We have to pick up useful information by reading the complex FTIR spectra even if only small changes occurred in the clinical specimens for diagnosis. As

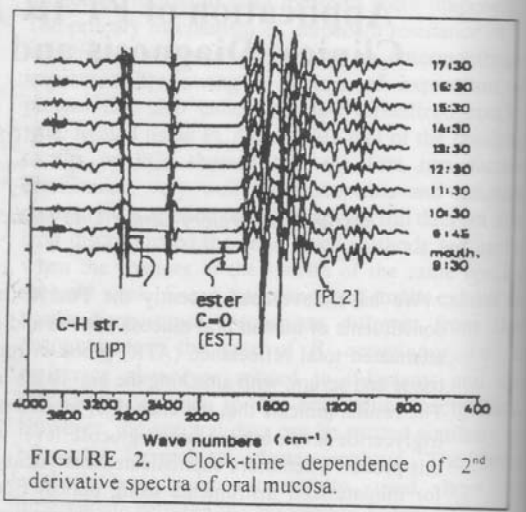

FIGURE 2. Clock-time dependence of 2nd derivative spectra of oral mucosa.

shown in the spectra (Fig.2), more than 20 bands were discernible when the second derivative spectra were measured with good signal-to-noise ratio. Normally the useful information about the change of clinical specimens may be obtained by comparing in detail several infrared band intensities and positions. However, this simple comparison task seems very tedious and takes a lot of time to accomplish it, and this task may not be actually applicable to the clinical diagnostic fields. For this reason we have to develop a technique to analyze and compare several infrared spectra instantaneously and accurately.

First I tried to use a statistical (multi-variate analysis) technique to compare several infrared bands after normalization of band intensities and analyze the spectral changes as a function of clock time and intake of meal. First I applied the factor analysis technique to the spectra. Fig.3 shows the clock-time dependent pattern of factor scores (Factor1 to 5), where those scores were obtained by the factor analysis. In this figure, the factor 1 which was closely related to the infrared bands originated from fatty acid methylene CH group increased only 2 to 3 hours later after the lunch intake. However, this pattern varied depending on the individuals and the content of meals. Clinical data suggested that normally the blood triglyceride content increased, depending on the individuals, nearly 1 to 2 or more hours later after the meal intake. The present infrared spectral data suggested that oral mucosal lipid content was affected significantly by the blood triglyceride level.

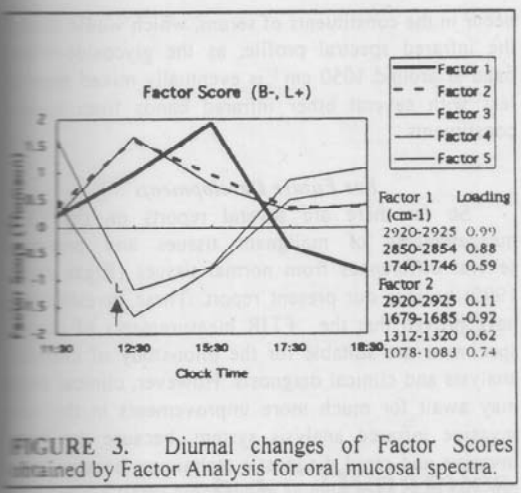

FIGURE 3. Diurnal changes of Factor Scores obtained by Factor Analysis for oral mucosal spectra.

The above factor-analysis method provided a reasonable result for the interpretation of mucosal infrared spectra. Previously another multi-variate analysis techniques were reported such as the partial least-squares (PLS) regression algorithm (3). However, besides these research purpose, we have to establish more sophisticated rapid data processing method such as the neural networks for clinical use.

Correlation of human serum glucose amount with change of infrared glycoside bands of serum

Using the same ATR probe as described above for in vivo oral mucosa measurement, I measured clinically obtained human sera. As the probe is rod-shaped (Fig.4),

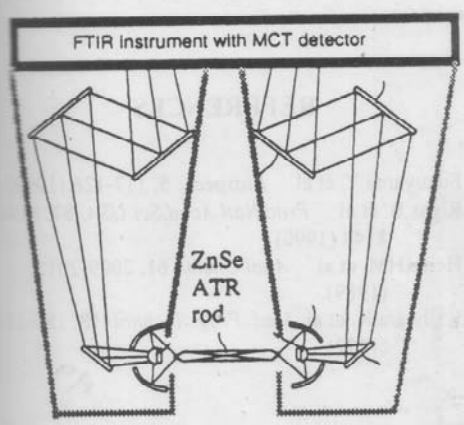

FIGURE 4. Top view of ATR adaptor for measurement of oral mucosa and serum using ZnSe rod.

the setting of a drop of serum solution was initially difficult. I improved this situation by wrapping the solution with aluminum foil tightly, and with this aluminum wrapping of less than 10 μL of the solution volume was sufficient to be measured and the sensitivity was practically acceptable. Initially I tried the wrapping of serum on the ZnSe rod with a 'Kimwipe' paper, but this did not provide good results, because the sample was dried up rapidly and a larger volume (nearly 100 μL) of serum was necessary. Moreover the sensitivity was not so high (nearly 1/3) as the case of aluminum wrapping.

With this technique, the infrared ATR spectrum of serum was obtained as shown in Fig.3 after subtraction of water spectrum. The reference spectrum was obtained for the aluminum-wrapped phosphate buffered saline. I tested the effect of coating of ZnSe rod probe by gold vapor deposition whether enhancement of infrared absorption (surface enhanced IR) occurred, however, there was not significant improvement in the infrared absorption of serum constituents. So in the measurement of serum I used only an aluminum foil wrapping of serum solution on the ZnSe rod.

Here I focus on the glycoside infrared bands at 3300 cm-1 originated from hydroxyl group of glycoside and at 1080-1050 cm-1 from C-O stretching mode. In the infrared spectral measurement, the glycoside-derived spectra were originated from many glycoside-containing materials, such as glycoproteins and glycolipids as well as free glucose or other hexoses. This would make difficult to compare directly the infrared glycoside spectra with serum glucose which is determined clinically with high specificity. Glucose has an infrared band at 1033 cm-1 (4), however, there was no clear band or even shoulder at 1033 cm-1 in the serum infrared spectrum.

Actually the correlation analysis (n=30) between

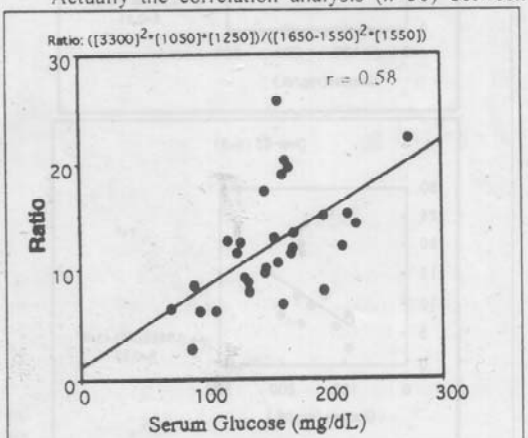

FIGURE 5. Serum glucose and infrared spectral ratios of serum.

serum glucose level (after clinical glucose-loading) and the serum infrared spectrum showed a good shape as shown in Fig.5 (r=0.58). Here a ratio was adopted in combination among bands at 3300, 1050, 1650, and 1550 cm-1, where the relative amount of glycosides against that of proteins was presented.

This significant correlation between blood glucose and infrared band intensity of glycosides in serum may indicate that the amount of serum glycoside compounds is affected by the blood glucose level, as the case of correlation between the amount of glycohemoglobin and blood glucose level. This may suggest that the infrared analysis of serum glyco-compounds indirectly reveals the change of blood glucose level, however, the analysis should be based on the accumulation of a lot of spectral data of individuals. As the infrared spectra of oral mucosa or other tissues are much more complex than serum, the correlation between blood glucose and mucosa spectrum would be dispiritedly low. Actually, as shown in Fig.6, when the test group was segregated into younger (below 60 years old) and older (over 65 years old) groups, the slope and regression coefficient were not different, but the Y-axis intersection of the regression line was larger in younger group than the older group, and this suggests that the plots diversity was affected by age. As the serum is a complex mixture of proteins, lipids, and glycosides, the age- and other individual factor-dependent changes may occur in the constituents of serum, which would change the infrared spectral profile, as the glycoside-related band at around 1050 cm^{-1} is eventually mixed more or less with several other infrared bands from various constituents.

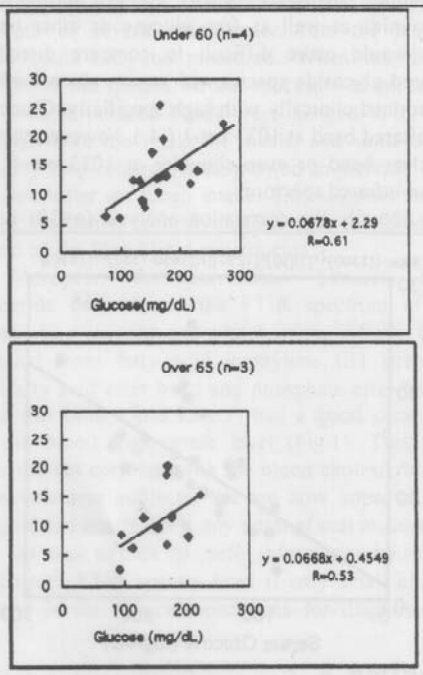

FIGURE 6. Correlations for younger and older groups.

For Future Developments

So far there are several reports on the FTIR measurements of malignant tissues and presented several differences from normal tissues (Rigas et al., 1990) besides our present report. These investigations have proved that the FTIR measurements of clinical specimens are suitable for the pilot-study of chemical analysis and clinical diagnosis. However, clinical fields may await for much more improvements in the non-invasive infrared analysis system, because the least-invasive and rapid diagnostic techniques become more popular than ever such as endoscopic small optical fiber screening, laparoscopic surgery system, and optical coherence tomography in clinical ophthalomology. Moreover, a rapid and accurate analysis technique for huge data set of infrared spectra must be developed especially in the clinically diagnostic field using advanced "chemometrics". Improvements of FTIR techniques to miniaturize the hardware system for personalized use and to obtain rapidly the diagnostic data would make advances in the "order-made" or "personalized" diagnostic system in the future.

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