In vivo and in situ detection of colorectal cancer using Fourier transform infrared spectroscopy

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INTRODUCTION
Cancer is one of the leading causes of death in the world. The mortality rate from malignant neoplasms has increased markedly in the last four decades. The important goal in cancer research is to develop an accurate, quick, convenient, and inexpensive cancer detection method to increase the survival probability.

Fourier transform infrared spectroscopy, as an effective tool for investigating chemical changes at molecular level, has been utilized to detect carcinoma[8-17]. This method has many advantages, for example, it possesses a promising perspective in detecting early cancer, which is very important for the survival of cancer patients. We have investigated the detection of malignant tissues, such as stomach, esophagus, gallbladder, colon, lung, liver, parotid gland carcinomas, etc. with FTIR spectroscopy since 1995[18]. Our research work consisted of three steps. The first step was to study the differences between malignant and normal tissues, which were stored in liquid nitrogen. The specimens were thawed at room temperature and measured in vitro using the FTIR method. In the second step of study, fresh samples obtained during surgical operation were measured by the FTIR method in vitro immediately. In these two steps of fundamental research, we demonstrated that the malignant tissues could be distinguished from the normal tissues measured in vitro using the FTIR method. The third step was the aim of this study, which was to measure the tumor in vivo and in situ using fiber optics with the FTIR method. FTIR fiber-optic technique can exhibit perspectives in cancer diagnosis in vivo and in situ because of its advantages[18].

Colorectal cancer is one of the most frequent cancers in Western countries[19]. It is the fourth leading cause of cancer deaths and tends to increase in China, especially in big cities like Shanghai. Reducing the mortality poses a big challenge for clinicians and researchers. It is of great importance to diagnose colorectal malignant tumors in vivo and in situ using the FTIR method and fiber optics with an ATR probe. Real-time and rapid identification of the malignant tissues would be performed during surgical operation. It is helpful for the surgeons to reduce the waiting time for the pathological results. Furthermore, it allows accurately and rapidly to determine the proper operative treatment, for example, the rapid determination of cut edges of surgically resected specimens, which is also a goal of surgery in the removal of neoplasms. The technique of FTIR with fiber optics has a promising perspective as a new non-invasive and early detection method of colorectal cancer.
MATERIALS AND METHODS

Patients and materials

Three colon cancer patients and one cecum cancer patient were measured in vivo using a FTIR spectrometer during surgical operation, and a rectum cancer patient was measured non-invasively in vivo and in situ before surgical operation in the Department of General Surgery, Third Hospital of Peking University, China. These five cases, including 4 males and 1 female, aged from 52 to 77 years (mean, 64.2 years), were analyzed in the present study. The consents of the patients were obtained before the experiments.

Spectral measurements

The spectra were measured in the Department of General Surgery, Third Hospital of Peking University by using a mobile WQF-500 FTIR spectrometer made in Beijing Second Optical Instrument Factory with a mid-IR fiber optics and ZnSe ATR probe (Spectra-Tech Corporation). A mercury cadmium telluride (MCT) detector was used and cooled by liquid nitrogen. Scans were performed with 4/cm resolution and 32 scans were co-added to increase the signal-to-noise ratio. Sterilization was strictly made at first.

The spectra of four colon and cecum cancer patients were measured in vivo and in situ during surgical operation. After strict sterilization, the ATR probe was put on the surface of the detected tissues by the surgeons and one spectrum was recorded for about one minute using a FTIR spectrometer. The spectra of the samples were collected from paired carcinomas and adjacent normal tissues in colorectal cancer patients. The FTIR measurement was non-invasive and harmless to the patients.

One rectum cancer case was measured non-invasively and in vivo before surgical operation. After strict sterilization, the fiber optics with ATR probe was inserted into the rectum through anus and put on the cancerous tissue 3 cm away from the anus. It took about one minute to measure the spectrum non-invasively. For the comparative analysis, the spectrum of the fresh tissue sample from the same site obtained during surgery was measured in vitro immediately.

Each FTIR analysis result was compared with the corresponding histological result, that is to say, the double blind method was used for FTIR and biopsy measurements.

RESULTS

For the systematic report of these colorectal cancer detection research projects, the detection of malignant colorectal tumor samples in vitro was introduced first. In our previous fundamental study, a large amount of frozen samples stored in liquid nitrogen and fresh tissues during surgical operation immediately were measured in vitro by using a FTIR spectrometer. The research results showed that there were obvious differences between the FTIR spectra of malignant and normal tissues measured in vitro as examples, the spectra are shown in Figure 1. Through the spectral analysis, the spectral characteristics of malignancy were as follows. The bands in the C-H stretching vibration in the region 2800-3000/cm and C = O band near 1700-1750/cm became weak and even disappeared. The peak of amide I band shifted to a lower wave number. The intensity of the amides II bands became weak, and the intensity of bands near 1400/cm was stronger than that of the bands near 1460/cm. The variations of the FTIR spectra between the normal and malignant colon tissues provided a basis and an opportunity for clinical application.

On the basis of the fundamental research, the FTIR spectra of malignant colon and cecum tissues measured in vivo and in situ during surgical operation were investigated. All the spectra of the measured colon and cecum cancerous tissues are shown in Figure 2. We could see that the relative intensity of 1446/1413 became smaller in the spectra of the malignant colon and cecum tissues. In addition, there was a weak amide II band near 1556/cm, a shift of the amide I band to a lower wavenumber near 1640/cm in the spectra of the cancerous colon and cecum tissues. The peaks of 2924/cm, 2989/cm, assigned to CH2 and CH3 vibration bands, and the band near 1740/cm related to C = O vibrations, became weak and even disappeared in the spectra of the malignant colon and cecum tissues. These spectral features indicated the malignancy of the detected tissues. The spectral characteristics and FTIR analysis results of all these cases are listed in Table 1. After the FTIR measurement, the detected colorectal tissues were resected and histologically verified as colorectal carcinomas. The FTIR analysis results were in agreement with the pathological results. Experimental results revealed that the spectral characteristics of normal and malignant colorectal tissues measured in vivo and in situ were similar to those obtained from in vitro measurement.

![Figure 1](image1.png)

**Figure 1** Typical FTIR spectra of colon tissues measured in vitro. A: spectrum of normal colon tissue sample; B: spectrum of malignant colon tissue sample.

![Figure 2](image2.png)

**Figure 2** FTIR spectra of malignant cecum and colon tissues measured in vivo and in situ during surgical operation. A: spectrum of malignant cecum tissue sample; B, C and D: spectra of malignant colon tissue samples.
Table 1 Results of FTIR spectral analysis and corresponding pathological detection of measured colorectal tissues

<table>
<thead>
<tr>
<th>Case number</th>
<th>Tissue</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Measurement date</th>
<th>FTIR measurement mode</th>
<th>FTIR spectra</th>
<th>FTIR analysis results</th>
<th>Pathological results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cecum</td>
<td>F</td>
<td>69</td>
<td>2003-07-21</td>
<td>in vivo during surgical operation</td>
<td>The bands of C-H stretching vibration and C = O vibration disappeared; peak position of amide I = 1 641/cm; $l_{\text{amide I}}$ (middle weak); $l_{\text{amide II}}$ (weak)</td>
<td>Adenocarcinoma</td>
<td>Carcinoma</td>
</tr>
<tr>
<td>2</td>
<td>Sigmoid colon</td>
<td>M</td>
<td>63</td>
<td>2003-07-22</td>
<td>in vivo during surgical operation</td>
<td>Colon cancer</td>
<td>Moderately differentiated adenocarcinoma of sigmoid</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sigmoid colon</td>
<td>M</td>
<td>52</td>
<td>2003-11-03</td>
<td>in vivo during surgical operation</td>
<td>Colon cancer</td>
<td>Carcinoma of sigmoid</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Transverse colon</td>
<td>M</td>
<td>77</td>
<td>2003-12-05</td>
<td>in vivo during surgical operation</td>
<td>Adenocarcinoma of transverse colon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Rectum</td>
<td>M</td>
<td>60</td>
<td>2003-07-22</td>
<td>in vivo before surgical operation</td>
<td>Rectum cancer</td>
<td>Moderately differentiated adenocarcinoma of rectum</td>
<td></td>
</tr>
</tbody>
</table>

In addition, one rectum cancer patient was measured non-invasively and in vivo before surgical operation. The spectrum before surgical operation was similar to that of the fresh tissue from the same site measured in vitro immediately (Figure 3). The C = O band near 1 740/cm, nearly disappeared in the spectrum as shown in Figure 3A. The band of amide I of protein was located at a lower wavenumber near 1 640/cm. The intensity of amide II bands located at 1 556/cm in the malignant tissue samples was less intense. The intensity of 1 469/cm band decreased. These spectral characteristics of the FTIR spectrum of malignant rectum tissue measured in vivo before surgical operation were consistent with those measured in vitro. The result of the FTIR detection for the rectum tumor was consistent with the biopsy test.

**Figure 3** FTIR spectra of malignant rectum tissues. A: spectrum measured non-invasively, in vivo before surgical operation; B: spectrum of fresh resected rectum tissue from the same site measured in vitro.

**DISCUSSION**

FTIR spectroscopy can provide information of molecular structure and composition. The development of cancer is always along the following sequences. Gene mutation is the first event, the second step is the alternation of biomolecules in both composition and molecular structure aspects. After that, the variation on cells and morphology of biological tissues will take place and can be detected by iconographic and pathological techniques. Vibrational spectroscopic methods, which are sensitive to the chemical changes at molecular level, may be developed as a powerful method to detect cancer at the second step of its development process, prior to most cancer diagnostic methods available today[25]

FTIR spectra are sensitive to the changes of biomolecules so as to diagnose the cancerous tissues[26]. The spectra of normal tissues often have a stretching vibration of carbonyl located near 1 745/cm, and the symmetry and asymmetry stretching vibrations of methylene located around 2 852/cm and 2 930/cm, as well as methyl at 2 873/cm and 2 958/cm. However, these peaks mentioned above often decrease, even disappear in the spectra of malignant tissues, because triglyceride contains a large proportion of methyl, methylene and carbonyl, and the fat in the region of the malignant tissue is consumed because of the increased nutritional and energy requirement in the development of carcinoma. The bands near 1 645/cm assigned to amide I of protein and deformation vibration of water molecule are located at a higher wavenumber in the normal colorectal tissue than those in the malignant tissue. The peak of amide II bands located at 1 545/cm in the malignant colorectal tissue is less intense and much broader than that in the normal tissue.

In conclusion, FTIR fiber-optic ATR spectroscopy can identify colorectal cancers in situ and in vivo. It provides real-time results for operating surgeons. FTIR spectroscopy can be applied to in vivo and in situ detection of not only colorectal cancer but also other malignant tissues of the digestive system. In addition, with further research, the technique of FTIR with fiber optics may exhibit its potential for non-invasive, in situ and in vivo detection of cancerous tissues before surgical operation. FTIR spectroscopic method with fiber optics
possesses a promising perspective to be a non-invasive, rapid, accurate and in vivo cancer detection technique.

REFERENCES

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