

Research Article

The Use of FTIR-ATR Spectrometry for Evaluation of Surgical Resection Margin in Colorectal Cancer: A Pilot Study of 56 Samples

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Colorectal cancer is one of the most common malignancies in human, and it is also one of the most leading causes of cancer-related death. Recently, Fourier transform infrared (FTIR) spectrometry is considered to develop into a new method for cancer diagnosis. In this study, colorectal cancer and mucosa 1 cm, 2 cm, and 5 cm away from tumor were measured by FTIR spectroscopy. FTIR spectra of colorectal cancer and mucosa 1 cm away from tumor were different from those of mucosa 2 cm and 5 cm away from tumor. According to the analysis of FTIR spectrometry, the decrease of lipid and the increase of protein and nucleic acid were observed in the former two sites. FTIR spectrometry, therefore, may be developed into a rapid promising method for judging surgical resection margin of colorectal cancer.

1. Introduction

Colorectal cancer is a disease with a major worldwide burden. It is the fourth most common malignancy with 1.2 million people developing colorectal cancer annually, and it is also the third most common cause of cancer death in the world, responsible for 0.6 million deaths annually [1, 2]. Surgical resection is one of the most important methods in the treatment of colorectal cancer. The range of surgical removal was directly related to the postoperative recovery and long-term survival outcomes of the patient. If the resection range is too small, it may lead to tumor recurrence after operation and influence the patient's long-term survival. And a beyond range of resection may lead to longer duration of postoperative recovery, even surgical complications. Therefore the determination of resection range is one of the core issues in the operation, and the safety of the distal and proximal resection margins of bowel should be ensured. Intraoperative pathological examination of frozen section is the most common method for checking the cutting edge,

which usually takes about 40 minutes one time. For some patients difficult to diagnose, it may need to be taken for pathological examination several times in the operation. It will prolong the operation time and cause adverse effect on the recovery of patients after operation.

Fourier transform infrared (FTIR) spectrometry provides abundant information concerning the variation of biological tissues at molecular level, so this approach may be developed as a new method for cancer diagnosis [3–9]. Meanwhile, less time is needed for each FTIR measurement, which takes approximately one to three minutes. In this paper, we perform a pilot study on the determination of surgical resection margin during the operation of colorectal cancer by using FTIR spectroscopic method coupled with optical fiber and attenuated total reflection probe. The aim of the present study is to develop a new method of FTIR spectroscopy as an intraoperative, rapid, and nondestructive diagnosing tool, which could determine the safety of resection margins in the colorectal cancer surgery.

2. Materials and Methods

2.1. Samples Collections. The study was approved ethically by Institutional Review Board of Peking University Third Hospital. All fourteen patients were fully informed with written consent. Fourteen fresh colorectal cancer specimens of radical resection were obtained from the Department of General Surgery in Peking University Third Hospital. In the operation room fresh samples were collected within five minutes after the colorectal specimens were removed. In each specimen four sites of fresh samples were obtained, which were shown in Figure 1 and recorded as Part A (tumor sample), Part B (intestinal mucosa sample 1 cm away from the tumor), Part C (intestinal mucosa sample 2 cm away from the tumor), and Part D (intestinal mucosa sample 5 cm away from the tumor). Fifty-six fresh samples were collected in this study.

Each sample was divided into two parts, approximately $0.5\text{ cm} \times 0.5\text{ cm}$ in size for each part. One was used for FTIR spectroscopic studies without any special sample pre-management. And the other part was processed as paraffin embedded blocks and sent to pathologists for pathological diagnosis.

2.2. Instrument. A WQF-660 FTIR spectrometer (Beijing Rayleigh Analytical Instrument Corporation, Beijing, China) equipped with a liquid nitrogen-cooled mercury cadmium telluride detector and an attenuated total reflectance (ATR) probe was used in this work. Freshly removed tissue was put on the ATR probe to collect FTIR spectrum. During the experiment, 32 scans were coadded to achieve an acceptable signal-to-noise ratio, with wavenumber ranging from 4000 cm^{-1} to 1000 cm^{-1} . All the spectra were recorded at a resolution of 4 cm^{-1} .

2.3. Data Processing and Statistics. FTIR spectra were measured using OMNIC E.S.P. 5.0 software (Nicolet Instrument Co., Madison, WI, USA). The following FTIR parameters were obtained: peak positions, full width at half maximum, and intensities, from which the relative intensity ratios were calculated. The above peak parameters were analyzed by using SPSS (version 17.0, SPSS Inc., Chicago, IL, USA), and normal distribution tests were performed in all parameters. For the normal distributed parameters, paired *t*-test was adopted. For the nonnormal distributed parameters, Wilcoxon paired signed-rank test was performed. $p \leq 0.05$ was considered statistically different.

3. Results and Discussion

3.1. Pathological Results. Pathological examinations with hematoxylin and eosin (H&E) staining were performed in all fifty-six samples. Adenocarcinomas were observed in all fourteen samples obtained in Part A and two samples in Part B. Adenocarcinomas were absent in the other twelve samples in Part B and all samples in Part C and Part D.

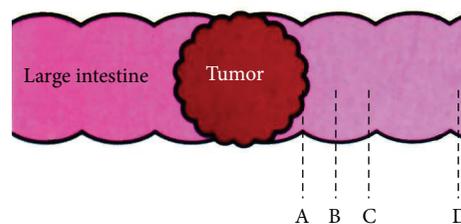


FIGURE 1: The schematic diagram of specimen of colorectal cancer removed and the samples obtained from four parts (Part A: tumor sample, Part B: intestinal mucosa sample 1 cm away from the tumor, Part C: intestinal mucosa sample 2 cm away from the tumor, and Part D: intestinal mucosa sample 5 cm away from the tumor).

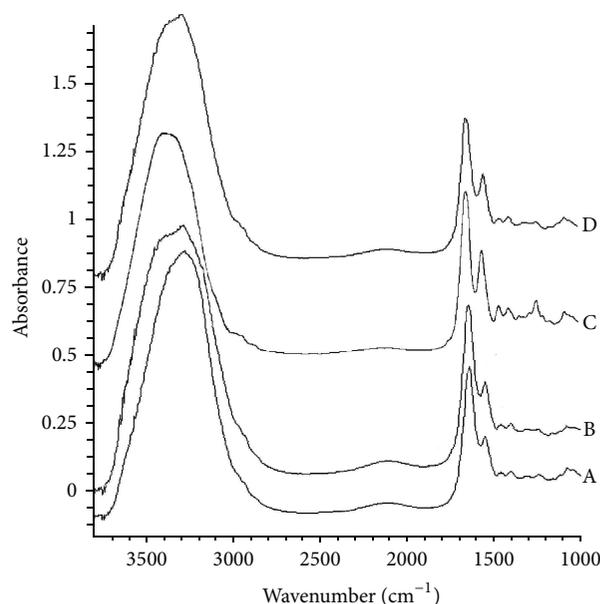


FIGURE 2: FTIR spectra of four samples from Parts A, B, C, and D, with wavenumber ranging from 3800 cm^{-1} to 1000 cm^{-1} .

TABLE 1: Preliminary assignments of Fourier transform infrared bands in colorectal cancer and mucosa.

| Wavenumber/ cm^{-1} | Preliminary assignment |
|------------------------------|--|
| 3275 | $\nu_{\text{O-H}}, \nu_{\text{N-H}}$ (water, protein) |
| 2925 | $\nu_{\text{as}}, \text{CH}_3$ (lipid) |
| 2855 | $\nu_{\text{as}}, \text{CH}_2$ |
| 1740 | $\nu_{\text{C=O}}$ (lipid) |
| 1640 | Amide I band (protein) |
| 1550 | Amide II band (protein) |
| 1460 | $\delta_{\text{C-H}}$ (lipid) |
| 1400 | $\delta_{\text{C-H}}, \delta_{\text{C-O-H}}$ (lipid) |
| 1303 | $\delta_{\text{C-H}}, \delta_{\text{C-O-H}}$, and amide III band |
| 1240 | $\delta_{\text{as}}, \text{PO}_2^-$ (nucleic acid) |
| 1160 | $\nu_{\text{C-O}}, \delta_{\text{C-O-H}}$, and $\delta_{\text{C-O-C}}$ (carbohydrate) |
| 1120 | $\nu_{\text{C-O}}, \delta_{\text{C-O-H}}$, and $\delta_{\text{C-O-C}}$ (carbohydrate) |
| 1083 | $\nu_s \text{PO}_2^-$ (nucleic acid) |

TABLE 2: FTIR spectrum comparisons of Parts A, B, C, and D (*t*-test results).

| Variable | Part A | | Part B | | Part C | | Part D | | <i>t</i> (<i>t'</i>) | <i>p</i> |
|---------------------|----------|-----------------|----------|-----------------|----------|-----------------|----------|-----------------|------------------------|----------|
| | <i>n</i> | Mean \pm SD | | |
| <i>I</i> / <i>I</i> | | | | | | | | | | |
| I_{1550}/I_{1080} | 14 | 5.25 \pm 1.26 | 14 | 5.43 \pm 1.03 | 14 | 6.83 \pm 1.34 | 14 | 6.56 \pm 0.89 | -2.631 | 0.001** |
| I_{1550}/I_{1460} | 14 | 5.93 \pm 1.42 | 14 | 5.60 \pm 1.35 | 14 | 4.84 \pm 1.08 | 14 | 4.67 \pm 1.20 | 2.216 | 0.041* |
| I_{1080}/I_{1460} | 14 | 1.08 \pm 0.43 | 14 | 0.95 \pm 0.33 | 14 | 0.65 \pm 0.27 | 14 | 0.71 \pm 0.22 | 2.095 | 0.036* |

I stands for the intensity of band; * $p < 0.05$; ** $p < 0.01$.

TABLE 3: FTIR spectrum comparisons of Parts A, B, C, and D (*rank-sum* results).

| Variable | Part A | | Part B | | Part C | | Part D | | <i>Z</i> | <i>p</i> |
|-----------------------------|----------|---------|----------|--------|----------|--------|----------|---------|----------|----------|
| | <i>n</i> | Median | <i>n</i> | Median | <i>n</i> | Median | <i>n</i> | Median | | |
| <i>P</i> / cm^{-1} | | | | | | | | | | |
| P_{2925} | 14 | 2918.3 | 14 | 2919.0 | 14 | 2927.2 | 14 | 2926.4 | -2.358 | 0.023* |
| P_{1080} | 14 | 1083.80 | 14 | 1083.8 | 14 | 1079.9 | 14 | 1078.40 | -2.179 | 0.032* |
| <i>I</i> / <i>I</i> | | | | | | | | | | |
| I_{1740}/I_{1460} | 14 | 0.42 | 14 | 0.44 | 14 | 0.55 | 14 | 0.51 | -1.074 | 0.065 |

P stands for the peak position of band; *I* stands for the intensity of band; * $p < 0.05$.

3.2. Spectra Analysis. There are 56 FTIR spectra obtained from 14 specimens. Four spectra of samples from one specimen were shown in Figure 2. In every spectrum, thirteen bands can be observed and the assignments of these bands are given in Table 1. After comparison, we found that the FTIR spectra from Part A of the 14 samples are similar to the corresponding FTIR spectra from Part B. FTIR spectra from Part C of the 14 samples are similar to the corresponding FTIR spectra from Part D. The differences between the four groups are shown in Tables 2 and 3.

3.3. The Variation of Infrared Bands Related to Lipid Contents among Different Parts of the Specimen. The vibration bands near 2925, 2855, 1740, 1460, and 1400 cm^{-1} were all related to lipid in the samples. The peaks around 2925 cm^{-1} in FTIR spectra of Part A and Part B undergo red shift in comparison with those in Part C and Part D ($p = 0.023$). The relative intensity of the 1740 cm^{-1} band to 1460 cm^{-1} absorption peak decreased in the spectra of Part A and Part B than in those in Part C and Part D ($p = 0.065$). The above results demonstrate that the lipid contents in Part A and Part B are less than those in Part C and Part D. According to the literature, the decrease of lipid bands in malignant part of tissues is related to the decrease of the content of fat. Two reasons may account for the decrease of fat in malignant tissues: normal tissues, including fat cells, are excluded by the proliferating malignant tissue in the growth of the tumor. Thus, less fat cells were found. The fat in the region of the malignant tissue is consumed because of the necessary increased nutritional and energy requirement in the development of the cancer [10].

3.4. The Variation of Infrared Bands Related to Protein among Different Parts of the Specimen. 1640 cm^{-1} absorption peak belongs to amide I band of protein and H-O-H deformation

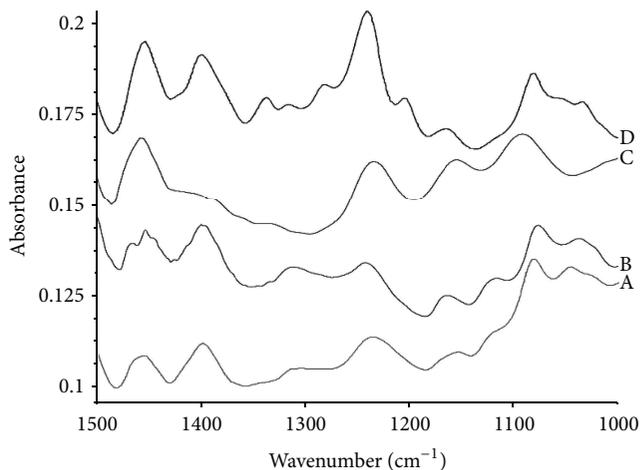


FIGURE 3: FTIR spectra of four samples from Parts A, B, C, and D, with wavenumber ranging from 1500 cm^{-1} to 1000 cm^{-1} .

vibration of water. 1550 cm^{-1} absorption peak arises from N-H bending and C-N stretching (amide II band) in proteins which may be related to the α -helix substructure of the protein. The relative intensity of the 1550 cm^{-1} band to 1080 cm^{-1} absorption peak decreases in the malignant tissue ($p = 0.001$). This may be caused by the increase of the nucleic acid or the decrease of the α -helix substructure of the protein in Part A and Part B. The ratio of the intensity of 1550 cm^{-1} peak to 1460 cm^{-1} peak was much higher in the spectra of Parts A and B than that in parts C and D ($p = 0.041$). This means that more protein is contained in the malignant tissue, which is produced from the faster cell metabolism [11].

3.5. The Variation of Infrared Bands Related to Nucleic Acid among Different Parts of the Specimen. 1240 cm^{-1} band

belongs to the symmetric P=O stretching band ($\nu_s(\text{P=O})$). 1080 cm^{-1} peak is assigned to the P-O-C stretching band ($\nu(\text{P-O-C})$). Blue shift of the 1080 cm^{-1} peak is observed in the spectra of Parts A and B in comparison with that in parts C and D ($p = 0.032$). The cell division during the reproduction of the tumor proceeds in an abnormal way and the structure of the nucleic acid may change. Thus the position of 1080 cm^{-1} peak in the spectra of Parts A and B undergoes blue shift. As shown in Figure 3, the ratio of the intensity of 1080 cm^{-1} peak to 1460 cm^{-1} peak was higher in the spectra of Parts A and B than that in Parts C and D ($p = 0.036$). This means that the relative content of nucleic acid to lipid increases in Parts A and B than that in Parts C and D. The reason is that tumor cell proliferation is faster in the malignant tissue; thus more nucleic acid is produced [12].

4. Conclusions

It can be concluded that FTIR spectra of colorectal cancer and mucosa 1 cm away from tumor were different from those of mucosa 2 cm and 5 cm away from tumor. FTIR spectra of colorectal cancer and mucosa 1 cm away from tumor were different from those of mucosa 2 cm and 5 cm away from tumor. According to the analysis of FTIR spectrometry, the decrease of lipid and the increase of protein and nucleic acid were observed in tumor and tumor surrounding area within 1 cm. FTIR spectrometry, therefore, may be developed into a rapid promising method for judging surgical resection margin of colorectal cancer.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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