Raman spectroscopic identification of normal and malignant human stomach cells

Jipeng Yang (杨继朋), Jianyu Guo (郭建宇), Liangping Wu (吴良平), Zhenrong Sun (孙真荣), Weiying Cai (蔡炜颖), and Zugeng Wang (王祖赓)

Key Laboratory of Optical and Magnetic Resonance Spectroscopy (East China Normal University), Ministry of Education; Department of Physics, and Department of Chemistry, East China Normal University, Shanghai 200062

Received May 13, 2005

Micro-Raman spectroscopy is employed to identify the normal and malignant human stomach cells. For the cancer cell, the reduced intensity of the Raman peak at 1250 cm^{-1} indicates that the protein secondary structure transforms from β-sheet or disordered structures to α-helical, while the increased intensity of the symmetric PO_2 stretching vibration mode at 1094 cm^{-1} shows the increased DNA content. The ratio of the intensity at 1315 cm^{-1} to that at 1340^{-1} reduces from 1.8 for the normal cell to 1.1 for the cancer cell in the course of canceration, and the ratio of the intensity at 1655 cm^{-1} to that at 1450cm^{-1} increases from 1.00 for the cancer cell to 1.26 for the normal cell which indicates that the canceration of stomach cell may induce saturation of the lipid chain.

OCIS codes: 300.6450, 170.5660, 170.0170, 170.1530.

Cancer has the second mortality in all diseases, and it is a serious disease threatening the people's health and life[1]. Thus, early diagnosis of cancer is crucial to effective treatment of the patients and to higher survival rates. However, the conventional morphological technique cannot detect accurately early cancer because of its unobvious symptom[2]. It is imperative to develop new techniques to diagnose various cancers at early stage, and then Raman and infrared (IR) spectroscopies have received more and more attentions for its larruping characteristics such as rich molecular structure information and non-invasion.

It is known that when normal cells or tissue turn into cancerous, the structural and configurative transformation of the biomolecules, such as DNA, lipid and protein, may occur[3−5]. Fourier transform infrared (FT-IR) spectroscopy is a sensitive analytical technique with the non-destructive advantage, which has been used to discriminate normal and malignant tissues and cells in the colon, breast, stomach, cervix, and skin basal cell carcinoma[6−12]. However, the strong IR absorption of water has strong effects on acquiring high-resolution and high-sensitivity IR spectra, and thus the tissues or cells should be inconvenience to be prepared and difficult to be measured *in situ*. Raman spectroscopy, a non-invasive and information-rich spectroscopic technique, can facilitate the structural determination of biological molecules in solid, solution or liquid state, and it can be used directly to measure biological samples *in situ* (such as cells, tissues, DNA, RNA, and protein) without labelling. During the last decade, much improvement has been made regarding Raman spectroscopy in molecular biology and pathology[2−5,13], and it has been used extensively to differentiate pre-malignant and malignant lesions from normal tissue in the brain, breast, gastro-intestinal tract, gynecologic tract, larynx, and skin[14−19]. However, to our knowledge, there have been no reports on micro-Raman spectroscopic identification of the normal and malignant human stomach cells.

Stomach cancer is rare in the developed countries, but it is an epidemic disease in the developing countries in South America and Asia. Presently, gastroscopy and histological examination are the primary methods for the clinic identification of stomach cancer. Gastroscopy can cause patients much pain, and unobvious symptom of the early patients often results in an erroneous diagnosis. Most histological examinations require histochemical staining and often require the use of several different staining methods. On the other hand, while performing fixation or staining, some specific property in specimen might disperse or be denatured. This calls for an advanced diagnostic technique in pathology that does not require fixation or staining. In this paper, micro-Raman spectroscopy is employed to discriminate normal and malignant human stomach cells with an expectation to use micro-Raman spectroscopy as a screening tool for stomach cancer.

Normal human gastric epithelia and human gastric adenocarcinoma cell were supplied by the Fourth Military Medical University, Shaanxi Province, and the Institute of Biochemistry and Cell Biology, Shanghai, respectively. Cells were cultured at 37 °C in a 5% CO_2 humidified incubator using a RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 50 ml HEPES, pH7.2, penicillin (100 u/ml) and streptomycin (100 mg/ml). Cells were centrifuged (3 min, 1500 rpm) and harvested,and then washed twice in distilled water and re-suspended in distilled water for Raman spectroscopy. The photograph for human stomach cancer cell is shown in Fig. 1.

Raman spectra were recorded by a confocal micro-Raman spectrometer (T64000, Jobin-Yvon, France), equipped with a 50-mW Ar-Kr laser excitation source at 514 nm, a microscope (IX 81, Olympus, Japan), a holographic notch filter to reject Rayleigh scattering, and a liquid nitrogen cooled charge-coupled device (CCD) detector (CCD-3000V, Edison N.J., USA). A 60× microscope water objective was used to focus laser and

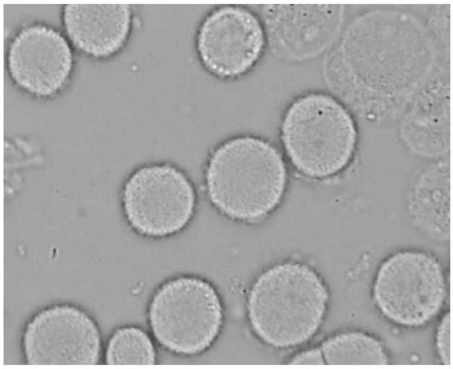

Fig. 1. Photograph for human stomach cancer cell (suspended in distilled water).

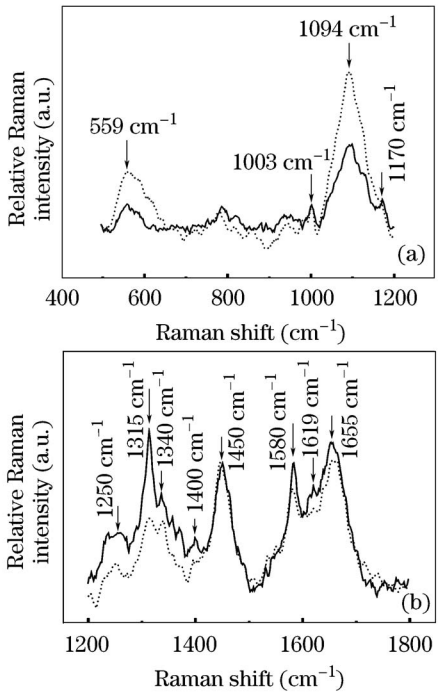

Fig. 2. Raman spectra of normal and malignant human stomach cells in the ranges of 500—1200 (a) and 1200—1800 cm^{-1} (b). Solid line: cancer, dashed line: normal.

collect Raman scattering on the cells. The spectrometer was calibrated with the silicon phonon mode at 520 cm^{-1}. Raman spectra in the range of 500—1800 cm^{-1} were recorded with an integration time of 60 s. The resultant Raman spectra were analyzed with Labspec6.0 software.

Raman spectra for normal and malignant cells in the range of 500—1800 cm^{-1} are shown in Fig. 2. The assignments of Raman bands are shown in Table 1. The spectra in the range of 500—1200 cm^{-1} are normalized by the peak at 1003 cm^{-1}[2], and that in the range of 1200—1800 cm^{-1} by the peak at 1450 cm^{-1}[3]. No significant frequency shifts are observed. As shown in Fig. 2(a), the band at 559 cm^{-1} is attributed to carbohydrate, and the band at 1003 cm^{-1} is assigned to the phenylalanine mode as an internal standard. The band at 1094 cm^{-1} is attributed to the symmetric PO_2 stretching vibration mode of DNA backbone, and it can be treated as an internal intensity standard for the DNA content[3]. In the course of canceration, the cells proliferate unlimitedly and DNA duplicates largely, which results in an increase of the intensity of the symmetric PO_2 stretching vibration mode at 1094 cm^{-1}.

Table 1. Assignments of Raman Bands of Cells

Band (cm^{-1})	Proteins	Band (cm^{-1})	Nucleic Acids
1003	Phenylalanine	1094	Backbone (PO_2)
1170	Tyrosine	1315	Base G
1250	Amide III	1340	Base A
1450	CH_2 Deformation		
1619	Tryptophan		
1655	Amide I		

As shown in Fig. 2(b), the band at 1250 cm^{-1} can be attributed to the amide III vibrational modes of the β-sheet and disordered conformations, and the reduced intensity indicates that the protein secondary structure transforms from β-sheet or disordered structures to α-helical in the process of canceration[3]. The band at 1400 cm^{-1} is due to the presence of lipid, and its intensity decreases as the lipid is ejected or digested in the course of malformation. The bands at 1170, 1619, and 1580 cm^{-1} can be assigned to tyrosine, tryptophan, and heme of the protein. These peaks are clearer in normal cells, which may suggest that the DNA content is less in normal cells than that in cancerous cells. Likewise, the band at 1315 cm^{-1} is attributed to guanine (G) and the band at 1340 cm^{-1} to adenine (A) of DNA. By a Gaussian fit in this region, it has been found that the ratio of the intensity at 1315 cm^{-1} to that at 1340 cm^{-1}, I_{1315}/I_{1340}, decreases from 1.8 for the normal cell to 1.1 for the cancer cell. The bands at 1655 and 1450 cm^{-1} can be attributed respectively to the amide I and CH_2 bending mode. It is reported that the intensity ratio of these two band areas can be used to differentiate normal tissue from abnormal one[4]. We have observed that this ratio increases from 1.00 for the malignant cell to 1.26 for the normal cell, which strongly suggests that canceration of the stomach cell may induce the decrease of the saturation of the lipid chain. So, Raman spectroscopy results indicate that the canceration of the stomach cell can lead to the structural and configurative transformation of the protein, nucleic acid, and lipid, which is consistent with FT-IR spectroscopic results[8,20].

Compared with the limited information obtainable from the study of tissues[21,22], the data derived from our study of cells provide rich and precise spectroscopic information.

In conclusion, Raman spectra for the normal and cancer stomach cells are identified and investigated. The study shows that in the process of canceration, the structures and configurations of protein, DNA, lipid, and their content undergo subtle changes, which may be effectively detected by Raman spectroscopy. The results clearly indicate that Raman spectroscopy has the potential application in clinical diagnosis for human stomach cancer, and provides useful information on the mechanisms of cancinogenesis.

This work was supported by the National Natu-

ral Science Foundation of China (No. 10234030 and 10374030), the National Key Project for Basic Research of China (No. 1999075204), the Shanghai Science and Technology Committee (No. 036105019), and the Twilight Project sponsored by Shanghai Education Committee (No. 03SG23). Z. Sun is the author to whom the correspondence should be addressed, his e-mail address is zrsun@phy.ecnu.edu.cn.

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