Characterizing lamina propria of human gastric mucosa by multiphoton microscopy

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Abstract. Lamina propria (LP) of gastric mucosa plays an important role in progression of gastric cancer because of the site at where inflammatory reactions occur. Multiphoton imaging has been recently employed for microscopic examination of intact tissue. In this paper, using multiphoton microscopy (MPM) based on two-photon excited fluorescence (TPEF) and second harmonic generation (SHG), high resolution multiphoton microscopic images of lamina propria (LP) are obtained in normal human gastric mucosa at excitation wavelength $\lambda_{ex} = 800$ nm. The main source of tissue TPEF originated from the cells of gastric glands, and loose connective tissue, collagen, produced SHG signals. Our results demonstrated that MPM can be effective for characterizing the microstructure of LP in human gastric mucosa. The findings will be helpful for diagnosing and staging early gastric cancer in the clinics.

1. Introduction

Due to poor diagnosis, the stomach cancer is the most second common cause of death [1]. Most stomach cancers develop in the gastric mucosa (inner layer or lining). The gastric mucosa consists of epithelial tissues, lamina propria (LP), and muscularis mucosae. The histologists and pathologists have been mainly focusing on epithelial tissue and LP which is the site that inflammatory reactions occur [2]. LP is composed of a delicate network of collagenous, reticular fibers and contains numerous capillaries, lymphocytes, some plasma cells, eosinophils, and mast cells. Because of the branching of the gland, crowding of the cells and the obscuring of the glands, it is often difficult to observe LP in human gastric mucosa.

Multiphoton microscopy (MPM) has many advantages over traditional optical imaging, including higher resolution, deeper penetration depth, inherent optical sectioning and less phototoxicity [3, 4]. In the nonlinear spectral imaging of the biosciences, MPM based on TPEF and SHG can be also used [3]. MPM was employed for characterizing human gastrointestinal mucosa at the cellular level without the

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need for fluorescent dye [5]. To the best of our knowledge, few studies were used to observe LP of human gastric mucosa by MPM. In this paper, the microstructure of LP in normal human gastric mucosa is investigated by using MPM.

2. Materials and Methods

In our study, the specimen was provided by the Surgical Department of Fujian Provincial Tumor Hospital. The fresh and normal gastric mucosa specimen was excised from the normal part of gastrectomy for the gastric cancer patient. In order to obtain a complete transverse cross-section of the LP in each section (5 μm in thickness) of normal human gastric mucosa, the mucosa specimen was excised perpendicular to the gastric layer. Theses sections were sandwiched between the microscope slide and the cover glass. To avoid dehydration or shrinkage during the whole multiphoton imaging, a little phosphate-buffered saline solution (PBS solution, PH=7.4) was dipped into the section specimen. Written informed consent was obtained from the patient prior to gastrectomy and our study protocol was approved by the ethical committee of Fujian Provincial Tumor Hospital.

The experimental setup of MPM has been described previously [6-8]. The excitation light source is a mode-locked femtosecond Ti: sapphire laser (110fs, 76MHz) tuning from 700 to 980 nm (Coherent Mira 900-F, Coherent Inc., Santa Clara, CA, USA). It was equipped through a laser scanning microscope (Zeiss LSM 510 META) with a Plan-Apochromat 63 × (N.A. = 1.4) oil immersion objective (Zeiss), which was used to focus the excitation bean and collect the backward signals. The polarization direction of the laser light is the horizontal polarization. The laser intensity attenuation was implemented by an acousto-optic modulator (AOM, Carl Zeiss Inc., Thornwood, NY, USA). By using a galvanometer-driven optical scanner (Zeiss), the excitation laser beam was scanned. A main dichroic beam splitter (MDFS, Zeiss, HFT KP 650) was employed for reflecting the incident excitation laser beam to the specimen and directing the TPEF and SHG signals to the META detector, which was used to detect the backward signals. The META detector is composed of a high-quality reflection grating as a dispersive element and an optimized 32-channel photomultiplier tube (PMT) array capable of collecting the dispersed output signal in 10.7 nm bandpass. All 32-channel PMTs cover a spectral width of approximately 340 nm across the visible spectrum (377-716 nm). In this work, the laser excitation wavelength is 800 nm. The META detector has eight detecting channels, and two independent channels (SHG and TPEF) from eight channels were used. One channel (387-409 nm) was used to detect SHG signal of collagen. Another channel (430-708 nm) was employed to detect TPEF signal of cells in gastric glands. Moreover, no photobleaching and photodamage were observed in a series of multiphoton imaging for LP within the normal human gastric mucosa. The images are 12-bit pixel depth.

3. Results and Discussion

Combining TPEF and SHG, high contrast and high resolution multiphoton images of LP in normal human gastric mucosa are obtained. Figures 1(a)-(c) display multiphoton images of LP in normal human gastric mucosa based on TPEF and SHG at excitation wavelength $\lambda_{\text{ex}} = 800$ nm. In Fig. 1(a), the cells of gastric glands in gastric mucosa can be visualized via TPEF (430-708 nm bandpass channel). The source of tissue TPEF signal is mainly the reduced pyridine nucleotides (NADH and NADPH; hereafter NAD(P)H) and oxidized flavoproteins (FPs) in the cells of gastric glands. As shown in Fig. 1(b), the connective tissue of LP can be imaged alone by SHG of collagen (387-409 nm bandpass channel). Overlaying two channels (SHG and TPEF), a higher contrast SHG/TPEF image of LP was obtained. As can be seen from the Fig. 1(c), the microstructure of LP in gastric mucosa can be readily observed. The gastric glands are separated by loose connective tissue bands. Furthermore, the glands are not quite straight and not quite parallel to one another.
To further characterize the LP in normal human gastric mucosa, the magnified multiphoton images were obtained, shown in Fig. 2. As can be seen, the microstructure of LP is more clearly observed. The green-fluorescing thick rope-like structures correspond to gastric glands in the gastric mucosa, whereas the red color signal originates from collagen of loose connective in the LP.

**4. Conclusion**

In conclusion, MPM based on TPEF and SHG was used to investigate the microstructure of LP in normal human gastric mucosa and high contrast and resolution multiphoton images of LP were obtained at excitation wavelength \( \lambda_{\text{ex}} = 800 \text{ nm} \). The results showed MPM is very effective to examine LP of normal human gastric mucosa at high contrast and resolution, without the need for additional fluorescent probe. Moreover, the method will be further used to examine the variation of LP in human gastric mucosa. Our results will be helpful for deciding the clinical diagnosis and the clinicopathological stage of gastric cancer. With the advance of endomicroscopy, MPM can provide the accurate and comprehensive information for the clinical research of human gastric tissue.

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