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Fiberoptic Confocal Raman Spectroscopy for Real-Time In Vivo Diagnosis of Dysplasia in Barrett's Esophagus

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Barrett's esophagus (BE) is a metaplastic precursor of esophageal adenocarcinoma (EAC). Since the late 1970s, despite extensive efforts for prevention (eg, periodic surveillance of high-risk BE patients), EAC still has had a substantial rise in incidence rates (>350%), and is growing more rapidly than other cancers in the developed countries.[1,2] Given the poor therapeutic response of symptomatic EAC, early identification of high-risk lesions (ie, dysplasia) together with therapeutic interventions is the most critical measures to improving survival rates of BE patients.[2] However, dysplastic lesions or grossly inconspicuous cancers are endoscopically indistinguishable from the surrounding benign tissue. This is because conventional endoscopy heavily relies on visual assessment of structural and morphologic changes of the tissue surface, resulting in poor diagnostic accuracy. Existing diagnostic guidelines recommend extensive biopsy samplings (typically 4-quadrant samplings) at every 1- to 2-cm interval along suspicious Barrett's segments during endoscopic inspections of BE patients. This approach produces a vast number of negative biopsies and is clinically labor intensive and a burden to the patients. Because only a minute amount of the mucosa is sampled (as little as 5%), tissue biopsies may not accurately characterize BE segments. Foci of dysplasia in a background of intestinal metaplasia are frequently overlooked, even when the biopsies are diligently performed by the experienced endoscopists using extensive 4-quadrant biopsy protocols. Taken into account the enormous rise in incidence rates of EAC and the existing clinical challenges, the need for new advanced endoscopic modalities has never been greater. The objective targeting of high-risk tissue areas (eg, high-grade dysplasia [HGD]) with a noninvasive or minimally invasive technique could greatly reduce random biopsy sampling errors as well as health care expenses on the patients. Recent attention has thus been directed toward molecular diagnosis using optical spectroscopy and imaging.[3] Raman spectroscopy represents a unique optical vibrational technique based on the fundamental premise of inelastic light scattering for tissue diagnosis and characterization.[4-6] When an incident laser light induces a polarization change of molecules, a small proportion of incident light photons ($\sim$1 in 10^8) is inelastically scattered with the frequency shifts corresponding to the specific Raman active vibrational modes of the molecules in the sample.[4] Taking advantage of the Raman spectroscopic ability of harvesting a wealth of fingerprint information from inter- and/or intra- cellular components (eg, proteins, lipids, and DNA) in cells and tissue, Raman technique has shown great promise for histopathologic assessments (ie, optical biopsy) at the biomolecular level.[5-8] In the last 2 decades, there has been accumulating evidence on the accurate diagnostic capability of Raman spectroscopy through comprehensive in vitro studies.[9] In vivo Raman endoscopic applications, however, have been limited not only by the difficulty in capturing inherently very weak tissue Raman signals, but also by the slow speed of spectral measurements (>5 s).[5,6] The miniaturization of flexible fiberoptic Raman probes with depth-resolving capability that can pass down the instrument channel of medical endoscopes for effective tissue Raman light collections presents another technical challenge in endoscopic applications of Raman spectroscopy.[6,7] To tackle these challenges, we have developed a novel beveled fiberoptic confocal Raman probe coupled with a ball lens capable of enhancing in vivo epithelial tissue Raman measurements at endoscopy.[7] We present this work on in vivo clinical applications of the fiberoptic confocal Raman spectroscopy for real-time objective diagnosis of dysplasia in BE at endoscopy. The direct assessment of the biomolecular contents of epithelial cells and tissue in vivo enables the gastroenterologists to perform noninvasive or minimally invasive optical biopsies in real-time during clinical endoscopy.

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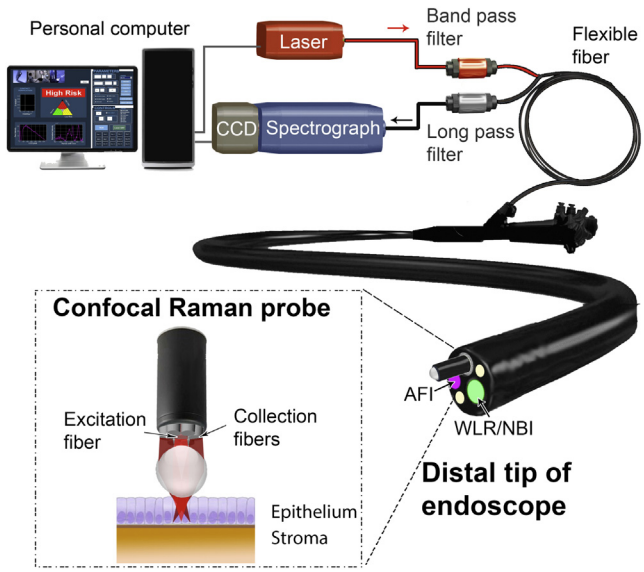

Figure 1. The rapid fiberoptic confocal Raman spectroscopy system developed for in vivo epithelial tissue diagnosis and characterization at endoscopy.

Fiberoptic Confocal Raman Instrumentation

The novel rapid fiberoptic confocal Raman spectroscopy technique developed for in vivo diagnosis of BE is shown in Figure 1.[6,7] Briefly, the fiberoptic confocal Raman spectroscopic system consists of a near-infrared diode laser (λ_{ex} = 785 nm), a high-throughput transmissive imaging spectrograph equipped with a liquid nitrogen-cooled, near-infrared–optimized charge-coupled device camera, and a specially designed 1.8-mm (outer diameter) beveled fiberoptic confocal Raman probe for both laser light delivery and in vivo tissue Raman signal collection.[7] The system acquires Raman spectra in the range of 800–1800 cm^{-1} with a spectral resolution of ~9 cm^{-1}. The 1.8-mm confocal Raman endoscopic probe comprises 9 × 200 μm filter-coated beveled collection fibers (NA = 0.22, beveled angle of ~20°) surrounding the central light delivery fiber (200 μm in diameter; NA = 0.22).[7] A miniature 1.0 mm sapphire ball lens (NA = 1.78) is coupled to the fiber tip of the Raman probe to tightly focus the excitation light onto tissue, enabling the effective Raman spectrum collection from the epithelial lining. Our Monte Carlo simulations indicate that approximately 85% of the Raman scattered light collected by the beveled fiberoptic confocal probe originates from the epithelium with an estimated tissue probing volume of <0.02 mm^3. The beveled fiberoptic confocal Raman probe can be inserted into the instrument channel of conventional endoscopes and placed in gentle contact with tissue surface for in vivo epithelial tissue characterization and diagnosis. The confocal Raman probe offers compelling advantages for clinical applications: (1) Selective targeting of the epithelial lining associated with early onset of Barrett's carcinogenesis is superior to conventional volume-type fiberoptic Raman probes that interrogate with a larger tissue volume (~1 mm^3); (2) a shallower tissue interrogation ability of confocal Raman technique suppresses tissue autofluorescence contribution and interference from deeper tissue layers (eg, stroma),[7] and (3) the reproducible and repeatable tissue Raman measurements are achieved in contact mode.[7] To fully utilize the above unique properties, we have integrated the beveled fiberoptic confocal Raman spectroscopy with multivariate analysis that enables epithelial molecular information to be extracted and analyzed in real-time in vivo.[6,10] The entire confocal Raman endoscopic system is controlled by customized software with auditory probabilistic feedback to the endoscopist, pushing the frontier of confocal Raman spectroscopy into routine clinical diagnostics.[10]

Survey of BE Using Confocal Raman Spectroscopy

The present study is part of a continuous nationwide study focusing on early diagnosis and treatment of gastro-esophageal malignancies by the Singapore Gastric Cancer Epidemiology, Clinical and Genetic Program, and conducted in the Endoscopy Centre at the National University Health System (NUHS), Singapore. This work was approved by the Institutional Review Board of the National Healthcare Group of Singapore. The trial was performed in accordance with International Conference on Harmonization for Good Clinical Practice guidelines, and the Declaration of Helsinki (2000). From August 2008 to June 2013, a total of 450 patients have been enrolled in Raman endoscopic examinations in Endoscopy Centre at NUHS, for surveillance or screening of various indications, including dyspepsia and neoplasia in the upper gastrointestinal (GI) tract. During endoscopic examination of suspicious lesions, each in vivo tissue Raman spectrum is acquired within 0.1–0.5 seconds, which permits a rapid survey of large tissue areas. Repositioning of the fiberoptic probe in the cases of poor Raman spectrum acquired can be realized using an online outlier detection method with auditory feedback to the endoscopist.[10] The in vivo Raman spectra acquired from 373 patients with different histologic subtypes in the upper GI have been used to construct a comprehensive Raman library (>12,000 Raman spectra). The tissue biopsies are subsequently taken from the tissue sites measured and sent for histopathologic examination by a group of GI pathologists in the Department of Pathology at National University Hospital. The pathologists were blinded to the results of confocal Raman scans. Biopsies are classified into the categories: Columnar-lined epithelium (CLE), nondysplastic BE defined as the presence of goblet cells, BE indeterminate for dysplasia, BE positive for low-grade dysplasia, and BE positive for HGD. The histopathology results (the gold standard) are compared with Raman measurements to determine the diagnostic performance of the confocal Raman technique for identifying dysplasia in BE.

We have developed a customized software to control the confocal Raman spectroscopy system for real-time data