Comprehensive imaging of gastroesophageal biopsy samples by spectrally encoded confocal microscopy

DongKyun Kang, PhD, Melissa J. Suter, PhD, Caroline Boudoux, PhD, Hongki Yoo, PhD, Patrick S. Yachimski, MD, William P. Puricelli, RN, Norman S. Nishioka, MD, Mari Mino-Kenudson, MD, Gregory Y. Lauwers, MD, Brett E. Bouma, PhD, Guillermo J. Tearney, MD, PhD

Boston, Massachusetts, USA

Background: Spectrally encoded confocal microscopy (SECM) is a high-speed reflectance confocal microscopy technique that has the potential to be used for acquiring comprehensive images of the entire distal esophagus endoscopically with subcellular resolution.

Objective: The goal of this study was to demonstrate large-area SECM in upper GI tissues and to determine whether the images contain microstructural information that is useful for pathologic diagnosis.

Design: A feasibility study.

Setting: Gastrointestinal Unit, Massachusetts General Hospital.

Patients: Fifty biopsy samples from 36 patients undergoing routine EGD were imaged by SECM, in their entirety, immediately after their removal.

Results: The microstructure seen in the SECM images was similar to that seen by histopathology. Gastric cardia mucosa was clearly differentiated from squamous mucosa. Gastric fundic/body type mucosa showed more tightly packed glands than gastric cardia mucosa. Fundic gland polyps showed cystically dilated glands lined with cuboidal epithelium. The presence of intraepithelial eosinophils was detected with the cells demonstrating a characteristic bilobed nucleus. Specialized intestinal metaplasia was identified by columnar epithelium and the presence of goblet cells. Barrett's esophagus (BE) with dysplasia was differentiated from specialized intestinal metaplasia by the loss of nuclear polarity and disorganized glandular architecture.

Limitations: Ex vivo, descriptive study.

Conclusions: Large-area SECM images of gastroesophageal biopsy samples enabled the visualization of both subcellular and architectural features of various upper GI mucosal types and were similar to the corresponding histopathologic slides. These results suggest that the development of an endoscopic SECM probe is merited. (Gastrointest Endosc 2010;71:35-43.)

The diagnosis of esophageal diseases is hampered by the inability of conventional video endoscopy to visualize pathologies such as dysplasia and early cancer in patients with Barrett's esophagus (BE) and eosinophilic esophagitis. Many approaches are being investigated to address this issue, including endoscopic microscopy or endomicroscopy, where high-resolution, microscopic images are acquired in vivo by using an endoscopic probe.[1,2] Confocal endomicroscopy is one technology that is capable of providing images that contain cellular and subcellular features of the esophagus.[3,4] In previous reports, confocal endomicroscopy has been shown to distinguish squamous mucosa from gastric pits,[5] BE with specialized intestinal metaplasia (SIM) from normal gastric or squamous epithelium,[6,7] and neoplasia associated with BE from SIM.[6-8]

Although confocal endomicroscopy shows promise, its field size is usually limited to less than 500 μm.[5,9] Thus, confocal endoscopic imaging may be subject to sampling

Abbreviations: BE, Barrett's esophagus; H&E, hematoxylin and eosin; HGD, high-grade dysplasia; MGH, Massachusetts General Hospital; OFDI, optical frequency domain imaging; RCM, reflectance confocal microscopy; SECM, spectrally encoded confocal microscopy; SIM, specialized intestinal metaplasia.

DISCLOSURE: *This research was supported by National Institutes of Health/National Cancer Institute (grant number R21CA122161). All authors disclosed no financial relationships relevant to this publication.*

0016-5107/$36.00
doi:10.1016/j.gie.2009.08.026

errors similar to those of endoscopic biopsy.[10] Mosaicing, or stitching, of multiple adjacent confocal images has been demonstrated to generate a considerably larger imaging area,[9,11] but even with these techniques, the total attainable field size is often significantly smaller than that of the mucosal surface area at risk, which can have a longitudinal extent of many centimeters.[12] A different form of endomicroscopy, termed comprehensive volumetric microscopy, has been proposed that acquires a 3-dimensional microscopic image of the entire distal esophagus. This approach has been demonstrated by using high-speed optical frequency domain imaging (OFDI) through balloon-centering catheters.[13,14] Although OFDI can provide architectural features of the imaged areas, cellular features are not well appreciated because of the 10- to 20-μm resolution of this technology.

An alternative reflectance imaging technology that has higher spatial resolution is reflectance confocal microscopy (RCM). RCM has been successfully used in imaging various types of human tissues, including esophageal,[15,16] gastric,[15,16] cervical,[17,18] skin,[19,20] joint,[21] and breast tissues.[22] In RCM, characteristic cellular and architectural features were rapidly visualized at video rates.[16,18,20,23] Although the image quality demonstrated in these RCM studies is very good, frame rates need to be increased further to comprehensively screen large luminal organ surface areas in living patients.

Spectrally encoded confocal microscopy (SECM) is a high-speed RCM technique that is capable of obtaining images at very high speeds.[24] With SECM, broadband or wavelength-swept narrowband light is coupled into a single optical fiber. Light from the fiber illuminates a grating and an objective lens at the distal end of the confocal probe. This configuration causes the sample to be illuminated at several different locations, where each point is interrogated by a different wavelength. After the light is transmitted back through the optics into the fiber, the image is reconstructed by detecting the returned light as a function of wavelength with a high-speed detector in the system's console. Because SECM does not need a rapid beam-scanning device inside the probe, the size of the optics can be small and the imaging speed can be very high, as much as 10 times faster than the video rate. These features of the SECM technology make comprehensive confocal endomicroscopy of the entire distal esophagus possible. The goal of this study was to investigate large-area SECM images of upper GI tissues and to provide a preliminary assessment as to whether these images contain sufficient microscopic information for the diagnosis of upper GI tract pathology.

Capsule Summary

What is already known on this topic

- Because spectrally encoded confocal microscopy (SECM) does not need a rapid beam scanning device inside the probe, the size of the optics can be small and its imaging speed high, making comprehensive confocal endomicroscopy of the entire distal esophagus possible.

What this study adds to our knowledge

- Large-area SECM images of gastroesophageal biopsy samples from 36 patients revealed subcellular and architectural features of various mucosal types that were correlated with histopathology.

METHODS

SECM bench-top system

A bench-top SECM system (Fig. 1) was used for the clinical study. A wavelength-swept laser (central wavelength = 1320 nm; bandwidth = 70 nm; repetition rate = 5 kHz)[25] was used as the light source. A transmission grating (groove density = 1100 lines/mm) and a water-immersion objective lens (effective numerical aperture = 0.7; focal length = 5.3 mm) were used to generate a single-scan field length of 180 μm along the spectrally encoded axis of the image. Two computer-controlled translational stages were used to scan the spectrally encoded line to obtain large-area images of biopsy samples. A piezoelectric transducer actuator was used to change the focal depth of the objective lens. The transverse resolution was measured to be 2 μm and the axial resolution was 10 μm. The image dimensions varied from 2 × 1 mm (4000 × 2000 pixels) to 5 × 3.6 mm (10,000 × 7200 pixels). The number of axial (depth) sections varied from 8 to 15 with axial scan intervals ranging from 15 to 10 μm, respectively, resulting in a total depth range of between 120 and 150 μm. The imaging time was between 2.5 and 15 minutes, depending on the biopsy sample size.

Patient enrollment and imaging procedure

Studies were conducted on biopsy samples taken from patients undergoing routine EGD at the Massachusetts General Hospital (MGH) Gastrointestinal Unit from June 2008 to February 2009. Any patient who underwent forceps biopsy was considered for enrollment, and biopsy samples from randomly selected sites from the esophagus and stomach were imaged. The biopsy samples were gently washed with saline solution. The first 14 biopsy samples were imaged in phosphate-buffered saline solution, and the remaining 36 samples were immersed in diluted acetic acid (0.6% concentration) to enhance nuclear contrast. The samples, with the epithelial surfaces preferably facing the objective lens, were placed under coverslips and then imaged in their entirety by the SECM bench-top system at 8 to 15 depth levels. Imaging began less than 5 minutes after biopsy of the patient. After imaging, the acetic acid–stained samples were washed in saline solution to reverse the aceto-whitening.[26] Each imaged