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Optical Biopsy in Human Gastrointestinal Tissue Using Optical Coherence Tomography

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Objectives: **Optical coherence tomography (OCT) is a new technique for performing high-resolution, cross-sectional tomographic imaging in human tissue. OCT is somewhat analogous to ultrasound B mode imaging except that it uses light rather than acoustical waves. OCT has over 10 times the resolution of currently available clinical high-resolution imaging technologies. In this work, we investigate the capability of OCT to differentiate the architectural morphology of gastrointestinal tissue with the long-term objective of extending OCT to endoscopic based diagnostics.** *Methods:* **Normal and diseased gastrointestinal tissues were taken postmortem and imaged using OCT. Images were compared with corresponding histology to confirm tissue identity and suggest the mechanisms that produce tissue contrast.** *Results:* **Microstructure was delineated in different tissues, including the esophagus and colon, at 16 ± 1 μm resolution, higher than any clinically available cross-sectional imaging technology. Differentiation of tissue layers, such as the mucosa, submucosa, and muscularis were achieved because of their different optical properties.** *Conclusions:* **The ability of OCT to provide high-resolution *in situ* imaging of gastrointestinal microstructure, without the need for excisional biopsy, suggests the feasibility of using OCT as a powerful diagnostic imaging technology, which can be integrated with conventional endoscopy.**

INTRODUCTION

High-resolution cross-sectional imaging technologies, such as high frequency ultrasound, have recently been developed to extend the diagnostic and therapeutic potential of endoscopic procedures. However, the relatively low resolution achieved with these imaging technologies, above 100 microns, has limited clinical utility (1–5). An imaging technology that can yield resolutions in the micron range can provide information on tissue microstructure that could only previously be obtained with conventional excisional biopsy. In this paper, we introduce optical coherence tomography (OCT) for obtaining high-resolution, cross-sectional images or "optical biopsies" of the gastrointestinal tract (6). The term "optical biopsy" refers to the use of optical techniques for performing *in situ* micron scale cross-sectional imaging of tissue without the need for excisional biopsy.

OCT is similar to B mode ultrasound imaging except that it measures reflected infrared light rather than acoustical waves. The intensity of backreflected light from structures within tissue is measured as a function of depth. Tomographic images are produced in a manner similar to radar, by scanning the optical beam across the sample and generating two-dimensional data sets. Thus, an OCT image represents a cross-sectional picture of the optical reflectance properties from within tissue. Although the penetration is limited to a few millimeters, the resolution of this optical technology is 5 to 25 times higher than high frequency ultrasound, the currently available clinical technology with the highest resolution for intraluminal imaging.

The first application of OCT involved imaging the transparent structures in the eye (7). Recent clinical studies have shown that OCT provides tomographic images of the retina with 10-μm resolution and can be used to diagnose a wide range of retinal macular diseases (8). However, the problem of OCT imaging in nontransparent human tissues is considerably more challenging because of optical scattering and attenuation. Recently, imaging in nontransparent tissue has been achieved by using longer wavelengths in the near infrared, taking advantage of the decreased scattering of light at these wavelengths (9–13).

In addition to the high-resolution, several features of OCT make it well suited for intraluminal diagnostics. Because OCT is based on mature technology used in optical communication, OCT can be constructed with common optical fiber components. Therefore, OCT imaging can be performed at sites within the gastrointestinal tract through optical fibers without the need for a distal transducer. Unlike magnetic resonance imaging or computed tomography, OCT is compact and portable. Finally, OCT does not require contact during imaging and can be performed directly through air without the need for a transducing media.

The purpose of this study is to investigate the capability of OCT to image the microstructure of the nontransparent tissue of the gastrointestinal tract. OCT imaging of gastrointestinal tissues was performed *in vitro* and correlated with histology. The results of this *in vitro* study suggest the feasibility of using

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OCT for high-resolution intraluminal imaging or optical biopsy of the gastrointestinal tract and serve as a foundation for future *in vivo* investigations. In particular, the high-resolution and fiber optic based design of OCT make it attractive for diagnostic imaging during endoscopic procedures.

MATERIALS AND METHODS

Normal and diseased gastrointestinal tissues, including the esophagus and colon, were obtained within 5 h of the initiation of autopsy. These tissue types have been examined because current *in vivo* imaging with high frequency ultrasound has met with limited success (14). More than 35 different samples from five patients were examined. The tissue samples were placed in isotonic saline with 0.05% sodium azide and stored at 0°C. The tissues were dissected to dimensions of approximately 10 × 5 mm and imaged with the luminal surfaces exposed. During imaging, the tissues were partially immersed in isotonic saline to prevent dehydration.

Imaging was performed through air at room temperature. The position of the beam on the sample was monitored using a visible light guiding beam (633-nm helium neon laser) that was coincident with the 1300-nm infrared OCT beam on the sample. The imaging planes were marked using small injections of dye. The samples then underwent routine histologic processing. Samples were immersed in 10% buffered formalin for 48 h. The tissues were then processed for standard paraffin embedding. Sections, 5 microns thick, were cut at the marked imaging sites and stained with hematoxylin and eosin or trichrome blue. The stained histologic sections enabled verification of tissue identity and in most instances allowed identification of sources of tissue contrast in the OCT images.

The principles behind OCT imaging have been described previously (6, 12, 15). OCT is similar to ultrasound except that it uses light rather than acoustical waves. In ultrasound, imaging is accomplished by measuring the delay time (echo delay) for an incident ultrasonic pulse to be reflected back from structures within tissue. Because the velocity of sound is relatively slow, this delay time can be measured electronically. However, since the velocity of light is 10^6 times higher than sound, OCT measurements of delay time, unlike ultrasound, cannot be performed directly by electronics. Therefore, a technique known as low coherence interferometry is used.

Figure 1 shows a schematic of the OCT system, which is based on a device known as a Michelson interferometer. Low coherence light, or light containing many different wavelengths, is generated from the source. The light is split evenly, half toward the sample and half toward a moving mirror. Light is then reflected both from within the sample and from the mirror. If the distance traveled by light in both arms is nearly identical, interference will occur when the light reflected from the sample and the light reflected from the reference arm mirror recombine at the beam splitter. More specifically, interference will occur if the two path lengths are matched to within a property of light known as the coherence length.

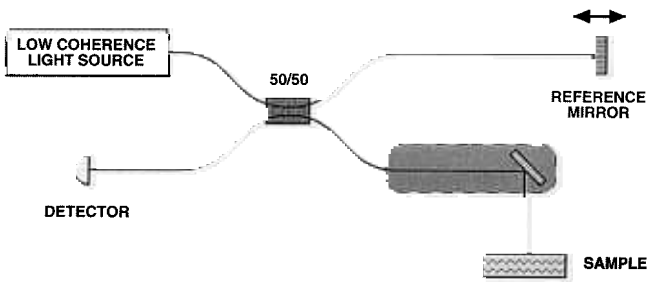

FIG. 1. Schematic of the OCT system. Infrared light generated from the low coherence source is split evenly, half to the sample and half toward a moving mirror. Light is reflected off the reference mirror and from within the sample and recombined in the beam splitter (50/50). The shaded area represents the portion of the system that would be present within the endoscope, consisting primarily of an optical fiber, lens, and mirror.

The position of the moving reference arm mirror is precisely controlled by the system electronics. Moving the mirror allows interference (backreflection) information to be obtained from different levels within the sample, because the distance traveled by light in the reference arm (mirror arm) is changing. Similar to A mode ultrasound, the intensity of backreflection is plotted as a function of depth. Only the sample arm, and not the reference arm or beam splitter, is present within the catheter or endoscope design (shaded area in Fig. 1).

The axial resolution of the OCT system is determined by the property of light referred to as the coherence length (12). The coherence length is inversely proportional to the bandwidth (wavelength distribution) of the source (12). The axial resolution of the 1300-nm superluminescent diode source used in this experiment was measured experimentally to be 16 ± 1 μm, which is not significantly different from the theoretically predicted resolution (based on the 50-nm bandwidth of the source) (12). The lateral or transverse resolution is determined by the spot size of the focused sample arm beam. The spot diameter is chosen so that it is comparable to the axial OCT resolution while maintaining an appropriate depth of focus (approximately 1 mm). A spot size of 25 μm was used in this experiment.

The position of the focused beam was scanned across the specimen, and axial profiles were digitized for each scan position to create a two-dimensional cross-sectional image. The dimensions of the OCT images in this study were 3 (axial) × 6 (transverse) mm, which corresponded to 250 (axial) × 500 (transverse) pixels. Thus, the digitized axial and lateral resolutions were 12 μm. The incident power on the sample was 150 μW, which provided a signal to noise ratio of 110 dB. The image acquisition time was 45 s.

RESULTS

Esophagus

OCT imaging of the esophagus allows visualization of the morphology of the mucosa and submucosa (Fig. 2). The upper portion of the mucosa appears homogenous in the OCT image. The muscularis mucosa is more highly reflec-

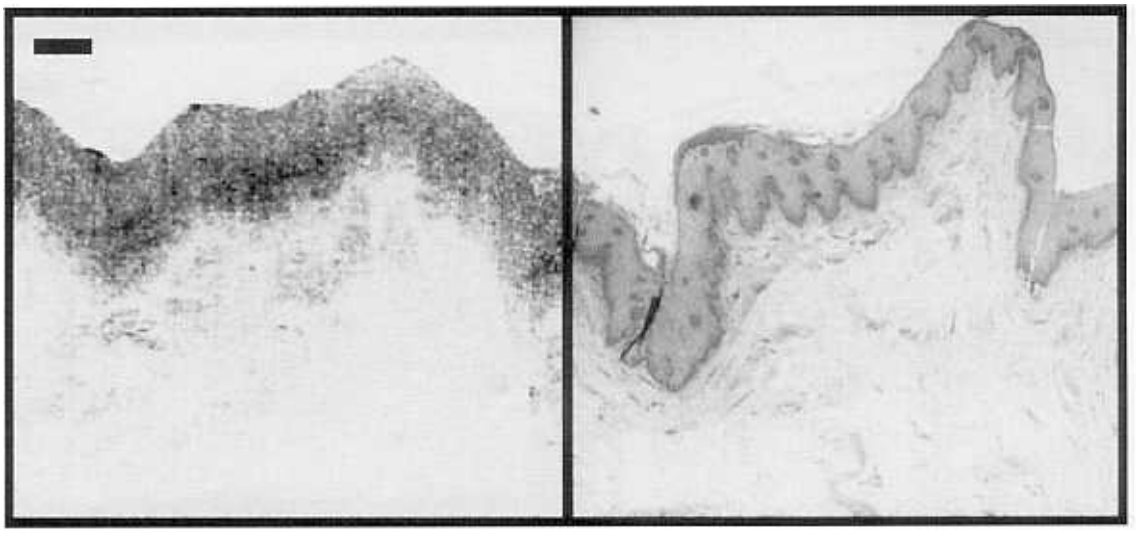

FIG. 2. Esophagus. OCT imaging of the esophagus (*left*) allows visualization of the morphology of the mucosa and submucosa. Bar represents 500 microns.

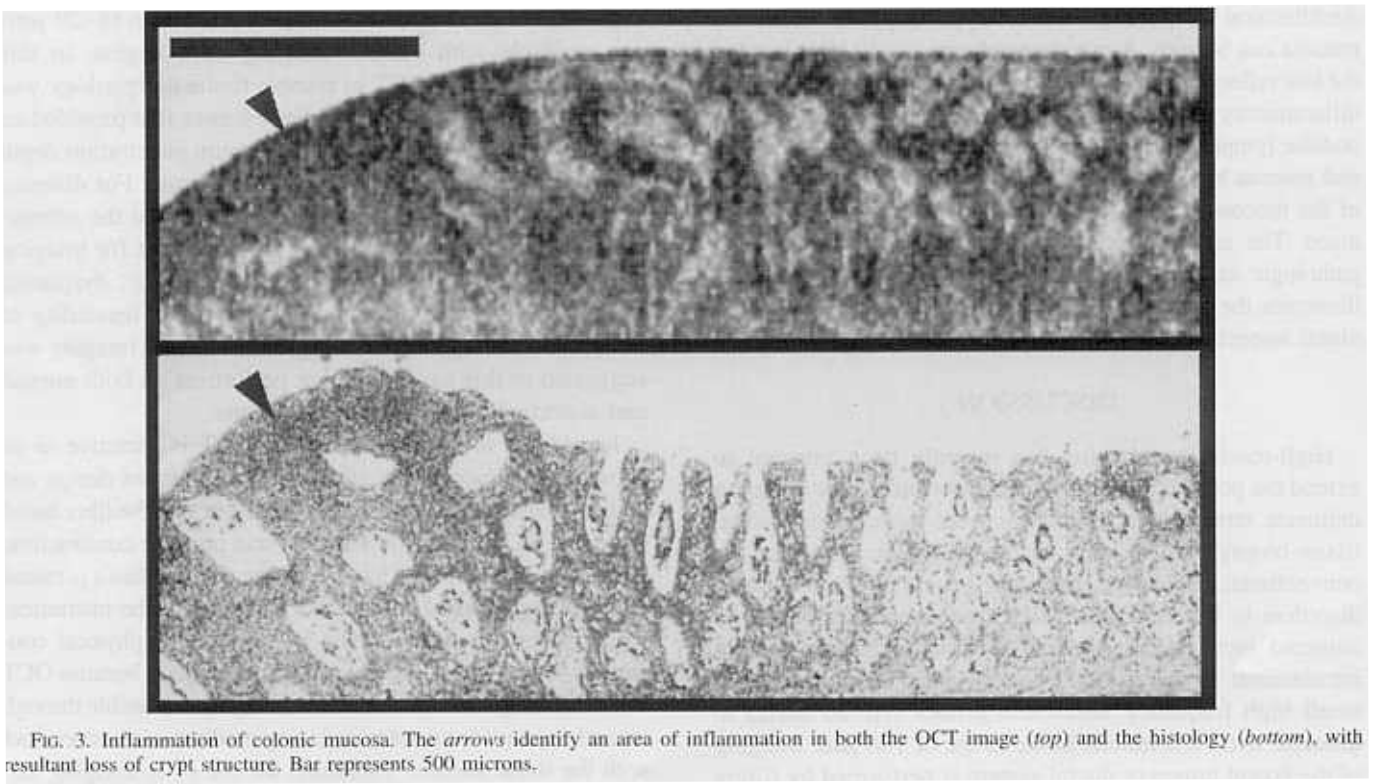

FIG. 3. Inflammation of colonic mucosa. The *arrows* identify an area of inflammation in both the OCT image (*top*) and the histology (*bottom*), with resultant loss of crypt structure. Bar represents 500 microns.

tive than the mucosa. A gap can be seen between the muscularis mucosa and the submucosa. Microstructure including veins can be seen in the submucosa as highly scattering foci. Surface folds in the histology image represent artifacts that occurred during histologic processing.

Colonic inflammation

Pathologic morphology can be visualized in OCT images of colonic inflammation (Fig. 3). Normal glands can be seen to the right of both the OCT image and the histology. These glands appear circular because the OCT system imaged these structures in cross-section. Inflammation is depicted by a high backscattering area in the OCT image (Fig. 3, *arrow*). Areas of hemorrhage, center, are noted in the OCT image as disruption of the normal mucosal morphology and resultant loss of crypt structure.

Colonic mucosa with pseudomembrane

This OCT image of the colon also demonstrates the capability of OCT to delineate pathologic microstructure (Fig. 4). The mucosa and muscularis mucosa can be differentiated due to the different backscattering characteristics within each layer.

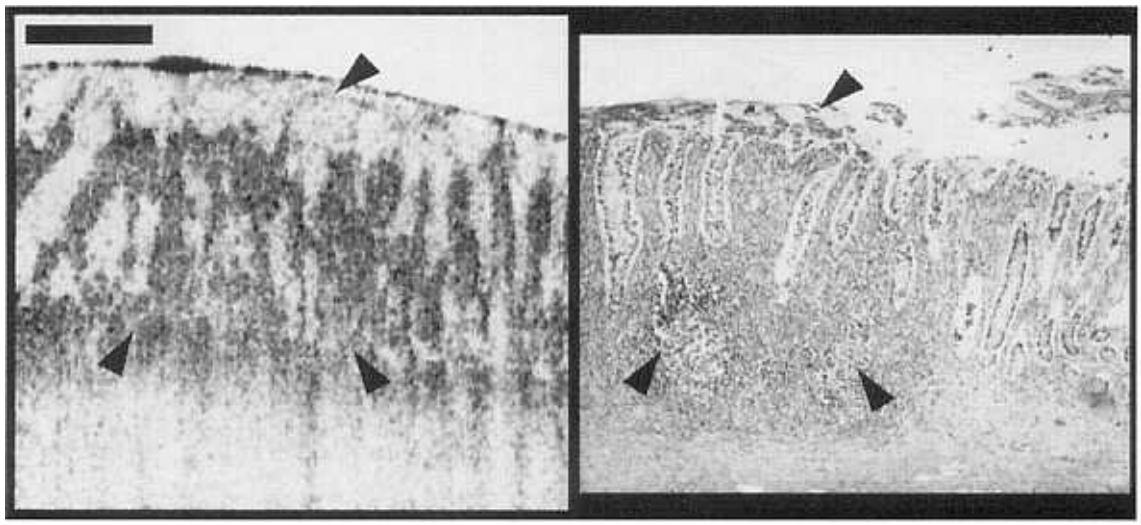

FIG. 4. Colonic pseudomembrane. *Top arrows* demarcate the colonic pseudomembrane in the OCT image (*left*) and the histologic specimen (*right*). *Bottom arrows* point to a nodular lymphoid infiltrate. Bar represents 500 microns.

Architectural morphology such as crypts or glands within the mucosa can be seen. A pseudomembrane can be visualized as the low reflectance layer overlying the mucosa proper. Deeper inflammatory lesions also distort the normal colonic glands. A nodular lymphoid infiltrate can be differentiated from the normal mucosa by its disruption of the normal linear morphology of the mucosal glands, causing the glands to appear disorganized. The initial clinical diagnosis of this lesion on gross pathologic examination was an adenoma. The misdiagnosis illustrates the limitations of diagnostic assessments based on visual inspection.

DISCUSSION

High-resolution imaging has recently been pursued to extend the potential of endoscopic procedures. The ability to delineate structure at resolutions near those of excisional tissue biopsy should improve the diagnostic limits of the conventional endoscope, allowing a wide range of clinical disorders to be addressed. High frequency ultrasound has attracted recent attention as a method of high-resolution intraluminal imaging (14, 16, 17). This technology uses small high frequency ultrasound probes (10–30 MHz) to generate axial resolutions in the range of 100 μm. Imaging of the bowel lumen or ductal system is performed by filling the lumen with saline or using a liquid filled balloon to couple the ultrasonic probe to the tissue surface. Ultrasound is used principally as an adjunct to endoscopy to diagnose and stage esophageal, gastric, pancreatic, and biliary neoplasms. However, because axial resolutions below 100 μm cannot be achieved, microstructure within tissue layers and cellular structure are poorly differentiated. Furthermore, the expense of the system, the relatively large size of the equipment necessary in the operative field, and the requirement of a transducing medium make ultrasound difficult and often impractical for routine integration with endoscopy.

OCT has the potential to allow the endoscopist to delineate tissue microstructure at an axial resolution (4–20 μm) not available with current imaging technologies. In this work, the ability of OCT to resolve tissue morphology was demonstrated with a 1300-nm diode source that provided an axial resolution of 16 μm. The maximum penetration depth of the OCT system was between 2 and 3 mm. For diseases that originate from or involve the mucosa and the submucosa, these first few millimeters are important for imaging the microscopic structure of early lesions (*e.g.*, dysplasia) and are well within the range of OCT. The feasibility of using OCT for high-resolution gastrointestinal imaging was suggested in this paper by work performed on both normal and abnormal clinically relevant tissue.

In addition to its high resolution, OCT is attractive as an adjunct to endoscopic imaging due to its compact design and ability to image through air. Unlike MRI or CT, the fiber based design of OCT allows for compact and portable construction, which can be engineered into a unit size smaller than a personal computer or standard defibrillator. The size of the instrumentation is particularly important in view of the physical constraints within the endoscopy suite. Furthermore, because OCT is based on light rather than sound, imaging is possible through air and does not require a transducing medium or direct contact with the tissue surface. Therefore, the use of an imaging balloon or saline injections, which can lead to impractical time demands on the gastroenterologist, is not necessary as it is with ultrasound.

Several issues will need to be addressed before the clinical effectiveness of OCT can be assessed, including improvements in data acquisition rates and integration with conventional endoscopes. The acquisition time for the images reported in this manuscript was 45 s. Future *in vivo* studies and ultimate clinical applications will require much faster acquisition. Recently, a prototype system has been developed with image acquisition times of 4 frames/s (250 ms/image) (18). Future modifications requiring straightfor-

ward, albeit time consuming, engineering including further improvements in mechanical scanning design and higher power light sources should enable near video speed (30 frames/s) image acquisition.

Because OCT is based on technology used in fiber optic communication, its integration into endoscopes is straightforward. Unlike ultrasound, no electronic ultrasonic transducer is required at the distal end of the endoscope because light can be transmitted bi-directionally through optical fibers, which are small and flexible. One possible endoscope for performing *in vivo* OCT imaging in the gastrointestinal tract could be similar in design to a recently developed OCT catheter for performing intravascular imaging (18, 19). This catheter directs the OCT beam radially and scans a circumferential pattern to generate a transluminal image. Sizes as small as 2.9 French (1 mm diameter) have been achieved and can be used to perform circumferential OCT imaging in conjunction with a standard GI endoscope. In addition, it should be possible to develop forward imaging OCT endoscopes that scan the OCT imaging beam in a determined pattern in the forward direction while providing operator viewing of the area being scanned. Finally, variable focus distal optics can also be incorporated for imaging gastrointestinal organ system lumen with different diameters.

Future modifications, which will likely improve the performance of OCT, will also focus on alterations in source wavelength and bandwidth. Both penetration and contrast in OCT images are dependent on the wavelength of the incident source. It has recently been demonstrated that imaging at 1300 nm allows a dramatic increase in penetration compared with 800 nm (10, 12). Examination of other wavelengths in the near infrared could yield further improvements in both penetration and contrast. The resolution of the OCT system is dependent on the bandwidth of the source. Recently, broad bandwidth, short-pulse femtosecond laser sources have been shown to yield resolutions in the range of 2 to 4 μm (20). Light sources with these broad bandwidth characteristics have the potential to provide the clinician with cellular level resolution.

In conclusion, we have demonstrated high-resolution, cross-sectional OCT imaging of several different gastrointestinal tissues *in vitro* and have correlated these images with conventional histopathology. The images acquired in this study provide information on tissue microstructure that could only previously be obtained with conventional excisional biopsy. Improvements in image resolution, acquisition time, and the development of a fiber optic endoscope are technically feasible and are the source of future investigations. These results suggest that OCT may become a powerful adjunct diagnostic imaging technology to conventional endoscopy, enabling "optical biopsy" or nonexcisional high-resolution diagnostic imaging, to be performed in gastrointestinal tissue.

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