ORIGINAL ARTICLE

Elastic scattering spectroscopy for the diagnosis of colonic lesions: initial results of a novel optical biopsy technique

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Background: Biopsy and polypectomy frequently are performed for lesions that carry a low risk of malignant transformation in the colon. Elastic scattering spectroscopy (ESS) is a novel optical biopsy technique that can distinguish, almost instantaneously, between normal and abnormal tissue in vivo, without the need to remove tissue. We assessed the diagnostic potential of ESS in the colon to differentiate normal colonic mucosa, chronic colitis, hyperplastic polyps, adenomatous polyps (with dysplasia), and adenocarcinoma.

Methods: ESS spectra were obtained from 138 sites in 45 patients at colonoscopy. They were then compared with conventional biopsy specimens taken from the same site, including normal colonic mucosa, hyperplastic polyps, adenomatous polyps, chronic colitis, and colon cancer. Spectral analysis was carried out with a validated computerized model that used principal component analysis followed by linear discriminant analysis. Cross validation was carried out by using 60% of the data as a ''training set'' and the remaining 40% of the data as a ''test set.''

Results: A total of 483 spectra were analyzed (290 normal, 19 hyperplastic, 69 adenomatous polyps, 74 chronic colitis, and 31 colorectal cancer). The sensitivity and the specificity of differentiating adenomas from hyperplastic polyps was 84% and 84%, respectively; for cancer from adenomatous polyps, 80% and 75%, respectively; for colitis from normal tissue, 77% and 82%, respectively; and for dysplastic mucosa (from polyps) from colitis, 85% and 88%, respectively.

Conclusions: ESS holds promise for differentiating colonic lesions with good accuracy and, therefore, is a potentially useful tool to make an instantaneous diagnosis during colonoscopy. It could prove a valuable aid for targeting biopsies in dysplasia surveillance in inflammatory bowel disease and for deciding which small polyps should be removed. (Gastrointest Endosc 2006;63:257-61.)

Despite significant technologic improvements in video endoscopy, conventional colonoscopy is unable to accurately differentiate hyperplastic polyps from adenomatous polyps or to diagnose dysplasia in a flat or polypoid lesion. This has led to the evaluation of a number of new optical/endoscopic imaging techniques such as optical-coherence tomography (OCT), light-induced fluorescence, Raman spectroscopy, elastic scattering spectroscopy (ESS), light-scattering spectroscopy (LSS), chromoendoscopy, magnification endoscopy, and narrow-band imaging.[1-4] These optical techniques are based on the interaction of light with tissue at the cellular and subcellular or even the biochemical level, in real time, thereby offering the potential of rapid diagnosis in vivo. For the technique reported here, a small optical-fiber probe is inserted through the biopsy channel of the endoscope; the tip of the probe is briefly placed in contact with the tissue spot under examination, and an instantaneous (a few milliseconds) optical measurement is made.

ESS is a point measurement technique that, when performed using appropriate optical geometry, is sensitive to the morphologic changes at that cellular and subcellular level. These include size and hyperchromaticity of cell nuclei, nuclear crowding, changes in the size of mitochondria, and other cellular organelles.[5] ESS spectra depend on the scattering efficiency of the cellular and subcellular organelles at each wavelength. Therefore, normal and abnormal tissues generate different spectral signatures, which represent the optical equivalent of histologic appearances. By using attributes such as light intensity ratios

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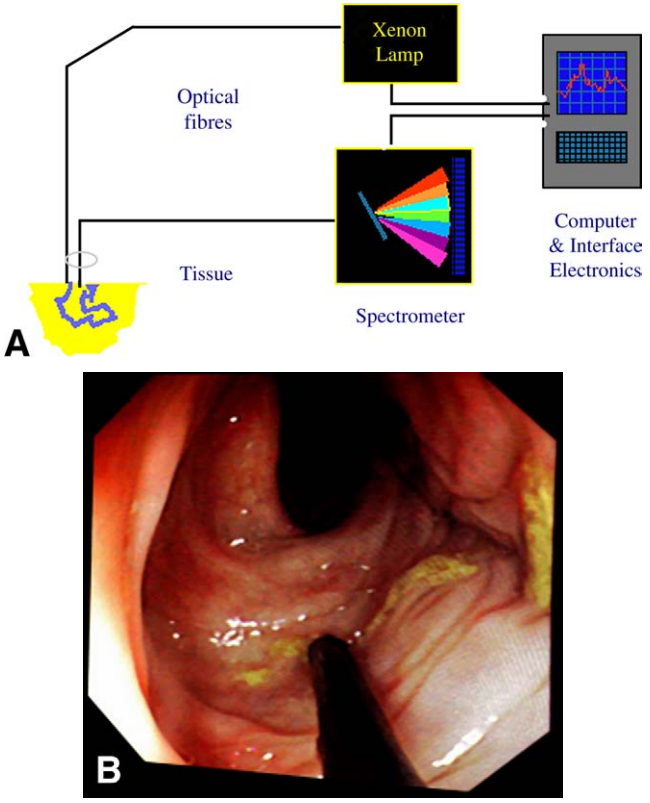

Figure 1. A, Schematic diagram of the elastic scattering spectroscopy system. **B,** The optical probe takes a measurement inside the colon during colonoscopy.

at various wavelength ranges, spectral data can be prospectively compared by using a variety of statistical computational analysis methods, such as principal component analysis and linear discriminant analysis, hierarchical cluster analysis, or neural-network pattern recognition.

The biologic basis for the use of ESS in the GI tract has been described, and it has been shown to have a high sensitivity and specificity for detecting dysplasia in Barrett's esophagus and for diagnosing breast cancer.[6-8] There have been only limited reports of its application for the differentiation of colonic lesions.[9] We report here our single-center experience of the use of ESS for the diagnosis of benign and malignant pathology in the colon.

PATIENTS AND METHODS

ESS equipment

The equipment used for ESS has been described.[10,11] Briefly, it consists of an optical biopsy box that contains the power supply, a pulsed xenon arc lamp and a spectrometer, an optical probe that contains the two fibers for transmitting and receiving light, and a laptop computer for spectral analysis (Fig. 1A and B). The xenon arc lamp[8] (Perkin Elmer, Inc, Fremont Calif) emits short pulses (~1 μs) of white light, between 320-920 nm, and

Capsule Summary

What is already known on this topic

- "Optical" biopsy techniques use the real-time interaction of light with tissue at the cellular and the subcellular level to provide a rapid, in vivo diagnosis.
- ESS is a novel optical biopsy technique that distinguishes normal from abnormal tissue in vivo, without the need for biopsy.

What this study adds to our knowledge

- In a pilot study, ESS showed a 77% to 88% accuracy in distinguishing various colonic abnormalities and has the potential to be used for targeting biopsies and for deciding which small polyps should be removed.

is coupled to one of the two optical fibers (400-μm diameter) to carry the light to the tissue. Ultraviolet B (280-315 nm) and ultraviolet C (100-280 nm) light is filtered out to avoid any potential risk to patients. A second optical fiber (200-μm diameter) collects the back-scattered light from the tissue to the spectrometer (S200; Ocean Optics, Inc, Dunedin, Fla), which analizes the spectrum between 300 and 800 nm by using a diffraction grating and charge coupled device array. The centers of the optical fibers are separated by 350 μm, and the two are encased in a plastic sheath to form an optical probe, approximately 2 mm in diameter and 300 cm in length, which can be passed down the biopsy channel of the colonoscope. The optical probe has a nontraumatic stainless steel tip that presents the optical fibers to the tissue at a fixed separation. The spectrum obtained is recorded and analyzed by the laptop computer that operates on a Microsoft Windows platform (Microsoft Corp, Redmond, Wash).

Spectral acquisition

Before any tissue spectra are taken, a white reference spectrum is recorded. This establishes the system response by recording the diffuse reflectance from a flat surface of Spectralon (Labsphere Inc, North Sutton, NH), which is spectrally flat between 250 and 1000 nm. The reference spectrum allows an accounting of spectral variations in the light source, the spectrometer, the fiber transmission, and the fiber coupling. To obtain a tissue ESS spectrum, the colonic mucosa must be free of surface debris, such as fecal matter or blood; if needed, a water jet is used to clean the mucosa. The optical probe then is placed in contact with the mucosa, perpendicular to the tissue, and a pulse of light is flashed onto it. The light is scattered through 180° by a series of elastic scattering events and is collected by the receiving fiber of the optical probe for spectral analysis by the spectrometer. Before the light flash is initiated, a "dark background" spectrum is recorded. This is subtracted to obtain the final spectrum.

TABLE 1. Sensitivity and specificity of elastic scattering spectroscopy in differentiating various colonic lesions

Category	Sensitivity, %	Specificity, %
All pathology vs. normal	92	82
Cancer vs. normal	80	86
Adenomatous vs. hyperplastic polyp*	84	84
Cancer vs. adenomatous polyp	80	75
Colitis vs. normal	77	82
Dysplasia vs. colitis	85	88

*Because of the small number of hyperplastic polyps, this result is not validated.

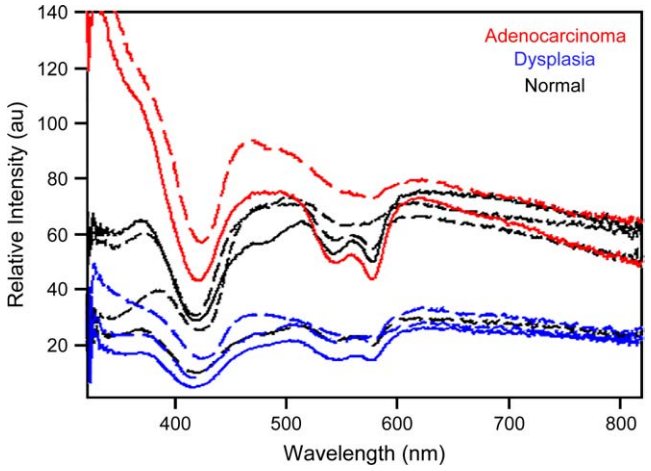

Figure 2. Comparison of the ESS spectral traces from different types of colonic tissue.

The entire process of spectral acquisition takes less than a second. In our institution, Olympus 200 series video colonoscopies (Olympus Corp, Keymed Ltd, Southend-on-Sea, UK) are used. These use a black and white chip system. Illumination is achieved by a strobing red/green/blue light source and by integrating the images through a processor. The strobing red/green/blue light interferes with data acquisition, therefore, the strobe effect is briefly suspended during optical biopsy measurement. This is done by changing the light output to a constant white illumination, which is the standard method of illumination for the Olympus 100 series and in most other conventional endoscopes of other manufacturers.

A single spectral measurement is able to interrogate a cylinder of tissue 1 mm^3 in volume. Three to 4 spectral traces were obtained from each site. The colonoscopist was careful to take a conventional pinch biopsy by using a standard biopsy forceps from the same site immediately after spectral acquisition. For polyps, the optical and the conventional biopsy specimens were obtained from the apex of the polyp; for cancers, nonulcerated areas were selected for examination.

Histopathology

All biopsy specimens were sent in individually labeled containers and were reported by an expert GI pathologist (MRN). Adenomas and dysplasia were reported according to the Vienna classification of GI epithelial neoplasia.[12] Chronic colitis was graded as mild, moderate, or severe; all adenocarcinomas, although pathologically graded as well, moderately, or poorly differentiated, were classed as a single entity for the purpose of this study.

Spectral analysis

All raw spectra were intensity corrected at 650 nm to allow better comparison. They also were visually examined by a physicist (KSJ) for any obvious outliers caused by acquisition errors, poor contact of the optical probe with the mucosa, or spectral contamination from fecal or extraneous matter; such spectra were excluded from subsequent analysis.

Principal components in the spectra were generated by using the statistics package SYSTAT 9 (Systat Software Inc, Point Richmond, Calif). To improve the accuracy of data analysis, 60% of the data were used to train the statistical algorithm, and the remaining 40% of the data were used as a ''test'' set. Linear discriminant analysis of the spectra was used to determine sensitivity and specificity of ESS to differentiate various colonic pathologies (Table 1). There were too few data for different grades of chronic colitis to be separately analyzed.

The study was approved by the institutional ethics committee of the University College London Hospitals, and written informed consent obtained from all patients.

RESULTS

A total of 138 colonic sites were sampled from 45 consecutive patients who were undergoing routine colonoscopy, and 483 ESS spectra were analyzed. This included 290 normal colonic spectra and 193 pathologic spectra (19 spectra from 4 hyperplastic polyps, 69 spectra from 23 adenomatous polyps, 74 spectra from 17 chronic colitis sites, and 31 spectra from 12 colorectal cancer sites).

The spectral differences between the ESS spectra obtained from normal colonic mucosa, an adenomatous polyp with dysplasia and adenocarcinoma, are shown in Figures 2 and 3. The difference in the intensities of the spectral traces in the region close to the ultraviolet wavelength range and the differences in the slope of the trace in the near-infrared region are evident. The sensitivity and the specificity of ESS in differentiating various colonic lesions is shown in Table 1. Because we did not detect any dysplastic lesion or mass (DALM) in any patient with

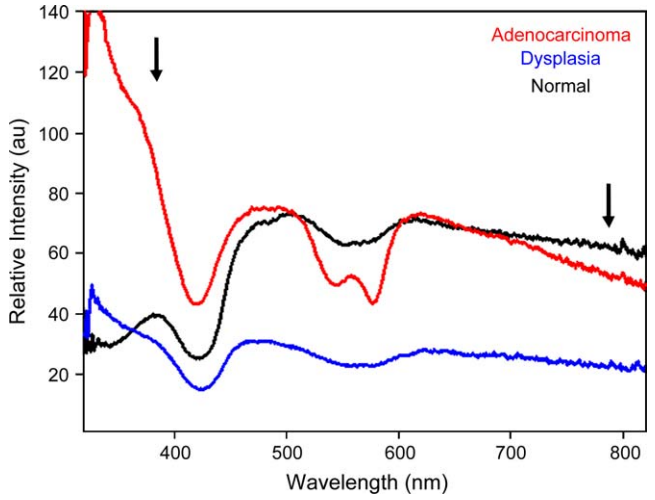

Figure 3. Representative spectral traces of colonic pathology, showing the differences in the peak intensity in the ultraviolet range (320-400 nm) and in the slope in the near infrared range (700-800 nm).

long-standing chronic colitis, it was not possible to evaluate the accuracy of ESS in this clinical setting. However, by assuming that the spectral characteristics of DALMs are similar to those of dysplastic mucosa in adenomatous polyps, we compared the spectra from chronic colitis sites with the adenomatous polyp spectra. The sensitivity and the specificity for differentiation was 85% and 85%, respectively.

If we assume a polyp prevalence of 10% in the general population, the positive predictive value and the negative predictive value (NPV) for ESS diagnosis of an adenomatous polyp are calculated to be 36% and 99%, respectively.

DISCUSSION

The recent advances in the endoscopic diagnosis of polyps and dysplasia in the colon have focused on two kinds of technologies: (a) advances in endoscopic optics and design for better resolution of mucosal detail, in combination with dye-spray chromoendoscopy, and (b) optical analysis techniques of cellular and subcellular detail by using spectroscopic methods, such as fluorescence spectroscopy, OCT, Raman spectroscopy, ESS, and LSS.

High-resolution magnification colonoscopy and pit-pattern diagnosis have been widely used in Japan and less often in the western world.[13] It is time consuming, technically demanding, and observer dependent; it also may be more reliable for the diagnosis of noncolitic dysplastic lesions. Methylene-blue- or indigo-carmine-aided high-resolution chromoendoscopy has been shown to detect 3 times more dysplastic lesions in ulcerative colitis surveillance, as well as in the detection of diminutive adenomas in noncolitic mucosa.[14,15] While these techniques are capable of detecting more lesions that otherwise may be missed at conventional colonoscopy, they still are unable to provide an instantaneous morphologic diagnosis of the nature of a polyp or the grade of dysplasia.

Optical techniques, on the other hand, provide additional information about the cellular and subcellular changes in a polyp or a suspicious lesion. Spectroscopic techniques such as laser-induced fluorescence, ESS, Raman spectroscopy, and optical coherence spectroscopy are all point-measurement techniques. To date, fluorescence spectroscopic techniques have prevailed in the colon with variable sensitivities and specificities (75%-95%) in diagnosing dysplasia.[16-21] Raman spectroscopy recently has been used to differentiate adenomatous polyps from hyperplastic ones, and a small in vivo study that involved 19 polyps reported sensitivity and specificity between 89% and 95%.[3]

Elastic scattering is a simple technology that is compatible with all endoscopy systems. We used it with an black and white chip system (Olympus), which interferes with our light collection. We were able to overcome this obstacle by briefly altering light output to constant white-light illumination. All color chip systems (e.g., Olympus 100 and Pentax systems [Pentax of America, Montvale, NJ]) are compatible, with no modifications.

The physics of elastic scattering is complicated. There is increasing evidence that cellular components with the highest refractive index, such as nuclear and subnuclear structures and bilipid membranes, cause most of the scatter.[22] Some studies of elastic scattering suggest that light scattering is probably sensitive to structures much smaller than nuclei, and most scattering probably occurs from internal structures with a mean diameter of only 0.2 μm within the nucleus. The degree of scatter has a complex association with the wavelength of light, and it is impossible to predict how a particular tissue with multiple organelles of varying sizes will scatter light.[1,23] Consequently, the practical use of ESS is dependent on statistical analysis of large numbers of spectra from normal and abnormal tissues.

In our present study, we have obtained sensitivity and specificity between 75% and 85% for the limited dataset examined. We also have been able to show a high NPV for the diagnosis of polyps, which means that a negative ESS spectrum almost certainly rules out an adenomatous polyp. We have shown that it is possible to differentiate hyperplastic from adenomatous polyps, chronic colitis from normal mucosa, and adenomatous polyps from cancer. The spectra from dysplastic mucosa of adenomatous polyps also are different from that of chronic colitis, and, therefore, it should be possible to diagnose dysplasia during surveillance for chronic ulcerative colitis. Because the technique relies on statistical analysis of data, the sensitivity and the specificity of ESS is likely to increase with larger numbers of spectra. Our center has been using ESS for the diagnosis of dysplasia in Barrett's esophagus for a longer time, and our preliminary results of 890 ESS spectra from 356 sites in 96 patients have shown a sensitivity for detecting dysplasia or cancer of 71% (68%-73%) and a specificity of 90%

(89%-96%) for a correct tissue diagnosis (neoplastic or normal).[7] The predicted NPV of the test was 96%.

ESS has some important advantages over other optical techniques. Firstly, the use of elastic instead of inelastic scattering (fluorescence or Raman spectroscopy) allows for a large optical signal, approximately 100 times stronger than fluorescence and 1000 times greater than Raman scattering. This makes the hardware needed for ESS cheap to produce and robust to use. Secondly, fluorescence spectroscopy involves the use of blue light, with bulky equipment, which is less user friendly than the ESS optical box. ESS equipment is compact and portable, and the technique is compatible with white-light colonoscopy. However, it is interesting to speculate that a combination of techniques may be more accurate than any single modality. Indeed, in a study[24] that combined fluorescence, reflectance, and LSS for diagnosing dysplasia in Barrett's esophagus, the individual techniques gave reasonable accuracy but, when combined, the sensitivity increased to 93% and the specificity increased to 100%.[24]

There are limitations to ESS measurements in vivo. Blood affects the spectra because of the absorption by heme. Taking an optical measurement from exactly the same spot as a conventional biopsy requires the colonoscopist to keep the colonoscope stable and still. A combined optical and conventional biopsy probe would resolve this problem. In its current format, ESS is an optical biopsy instead of an imaging tool. Others have demonstrated that it should be possible to extend this technology to image large areas of the mucosa.[25] However, this approach needs further development.

In conclusion, we have shown that ESS offers the potential of providing an instantaneous in vivo diagnosis of colonic polyps. There also is promising preliminary data that is evidence that it can differentiate normal mucosa from inflamed and cancerous mucosa. It, therefore, may become a useful tool to target polypectomy and in surveillance for dysplasia in chronic ulcerative colitis.

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