Gastrointestinal tissue diagnosis by laser-induced fluorescence spectroscopy at endoscopy

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An endoscope-compatible, optical fiber system has been developed which can be used to obtain laser-induced fluorescence spectra of mucosal abnormalities during endoscopy in real time. The results of our previous studies have suggested that laser-induced fluorescence tissue spectra are sufficiently unique that they can be used to accurately diagnose mucosal abnormalities in some systems. To test this hypothesis in vivo, laser-induced fluorescence spectra were obtained during colonoscopy from 31 colonic adenomas, 4 hyperplastic polyps, and 32 examples of normal mucosa in 20 patients. The resulting spectra could be used to correctly differentiate adenomas from normal colonic mucosa and hyperplastic polyps in 97% of the specimens studied with the results sensitivity, specificity, and positive predictive value of 100%, 97%, and 94%, respectively. These results, although preliminary in nature, suggest that laser-induced fluorescence spectra can be used in the recognition and differential diagnosis of mucosal abnormalities at endoscopy. (Gastrointest Endosc 1990;36:105-111)

Endoscopic diagnosis is predicated on the gross morphologic characteristics of gastrointestinal and colonic abnormalities. A reasonably accurate appraisal of the true pathologic nature of many lesions can be made by endoscop ic observation alone, but there remains a margin for error that can be substantial for certain types of lesions. Microscopic assessment of biopsy specimens is therefore necessary for many lesions discovered at endoscopy. Furthermore, certain abnormalities of a microscopic nature, dysplasia in chronic ulcerative colitis or Barrett's esophagus, for example, are usually unrecognized by gross endoscopic observation. For these reasons, an accurate method of tissue differentia tion or diagnosis that could be employed at endoscopy would be highly advantageous.

Low-power laser illumination can induce endogenous tissue fluorescence (autofluorescence) with spectral characteristics that depend upon physical chemical composition of the tissues. Since such fluorescence emission and attenuation (reabsorption and scattering) can be measured, this inherent property of tissue might be the basis of a diagnostic system that could be adapted to endoscopy. We have demonstrated that autofluorescence can be used to differentiate adenoma from normal mucosa in vitro using tubular adenomas from patients with familial adenomatous polyposis as a model system. This work demonstrated that the excitation wave length 370 nm was optimal for in vitro discrimination of adenomas from normal tissue, whereas the excitation wave lengths 330 and 430 nm could also be used. The results of these studies support the basic premise that stimulation of tissue with specific wave lengths of light results in tissue autofluorescence that is characteristic for a given tis-
sue. The purpose of this study was to test this hypothesis in vivo using the colonic adenoma as a model.

The colonic adenoma provides a convenient model with definite endoscopic and pathologic differences between normal and abnormal (adenomatous) tissue. The use of this model also allows for certain predictions, based on previous in vitro studies, concerning the optimum excitation wave length. Laser-induced fluorescence (LIF) spectroscopy may also have clinical relevance in relation to the problem of the “adenoma-carcinoma” sequence that is thought to underlie the development of colon cancer.\(^4\) \(^5\) \(^6\) It is well established that endoscopic differentiation of adenomatous from nonadenomatous polyps is not possible in the case of small (diminutive) lesions.\(^7\) \(^8\) Management of these polyps may be problematic in a number of ways. A biopsy is necessary for accurate diagnosis, but should a lesion prove to be an adenoma it may be difficult to locate it a second time for definitive treatment. The immediate treatment of diminutive polyps upon discovery means that patients with nonadenomatous lesions incur an unnecessary, albeit small, risk of a complication\(^9\) as well as the additional cost of treatment. Although LIF spectroscopy may refine the management of small colon polyps, the method has a further potential with respect to the diagnosis of other mucosal disorders that are more difficult to recognize endoscopically. The benign adenoma is analogous in certain respects to dysplastic epithelium found in other conditions so that it is possible that information derived from LIF spectroscopy of adenomatous tissue might be applied to the recognition of dysplastic mucosa in disorders such as Barrett’s esophagus or chronic ulcerative colitis.

**MATERIALS AND METHODS**

We have developed a unique spectrofluorometry system to collect mucosal fluorescence spectra in vivo (Fig. 1). A special optical fiber fluorescence probe was constructed that could be passed through the accessory channel of a standard colonoscope. The probe delivers monochromatic light at 370 nm produced by a nitrogen-pumped dye laser (Laser Science VSL-337 ND nitrogen laser and DLM-220 dye laser module) through a centrally placed optical fiber. This light forms a 1-mm diameter excitation spot at the distal tip of a 1-mm diameter transparent quartz outer shield. This system can be used to deliver excitation light spanning the spectral region 250 nm to 1.9 pm. At 370 nm excitation, the laser furnishes an average power of 270 µW at the distal tip, delivered in 3-nsec pulses at 20 Hz. Nine smaller peripherally placed optical fibers surround the central excitation fiber. These collect the emitted tissue fluorescence from the surface area directly illuminated by the excitation light. The system thus has a well-defined excitation and collection geometry.\(^10\)

The proximal ends of the nine collection fibers were imaged at the entrance slit of an imaging spectograph (Instruments SA CP-200) coupled to a gated optical multi-channel analyzer (EG&G PAR OMA III) under computer control. A 399-nm long-pass, low fluorescence filter was used to block scattered excitation light from the detector. A 1.4-msec collection gate synchronized to the laser pulse effectively eliminated the effects of the colonoscope’s white illumination light during collection of the weaker tissue fluorescence.

The patients studied with this spectroscopic system were selected from a group of patients referred for follow-up colonoscopy for suspected colonic adenomas seen at flexible sigmoidoscopy or with barium enema. Informed consent was obtained from each patient and the project was reviewed and sanctioned by the Institutional Review Board of The Cleveland Clinic and The Committee on Use of Humans as Experimental Subjects at The Massachusetts Institute of Technology.

The patients were prepared for colonoscopy by ingestion of an oral lavage solution (Colyte\(^\text{®}\)). We have tested Colyte\(^\text{®}\) in vitro and have shown that it does not interfere with LIF spectra collection. Intravenous sedatives and analgesic drugs, most often midazolam (1 to 2 mg) and meperidine (50 to 100 mg), were administered at the start of the procedure.

Colonoscopy was performed in the usual fashion using a standard colonoscope (Olympus CF-1T201). In each patient, the spectroscopy probe was passed through the accessory channel of the colonoscope and its outer shield was placed in direct contact with the surface of mucosal polyps and/or control nonpolypoid normal appearing mucosa. Direct contact displaced residual colonic contents and/or mucous. Direct
contact was also necessary to fix the distance between the mucosa and the distal end of the probe's optical fibers, so that reliable calibrated fluorescence intensity information could be obtained.

Fluorescence emission spectra were collected from 350 to 700 nm, with a resolution of 0.6 nm. After three spectra were obtained, the probe was removed and then replaced two to four additional times with three spectra obtained at each placement. This process yielded 9 to 15 individual spectra per site. No appreciable fluorescence photobleaching was observed. A biopsy for histologic examination was then performed of the mucosal site analyzed by the probe. Polyps were treated by standard electrosurgical snare polypectomy or coagulation biopsy (“hot biopsy”) in the case of diminutive polyps. Tissues were categorized histologically as normal, hyperplastic polyp, tubular adenoma, tubulovillous adenoma, villous adenoma, or as tissue insufficient for diagnosis. Biopsy specimens from 12 areas yielding lymphoid aggregates or tissue fragments insufficient for diagnosis were eliminated from the study.

Spectra were corrected for non-uniform spectral response of the detection system by using a calibrated lamp. The fluorescence intensity of a standard fluorescence paper was measured prior to study in each patient and was used to calibrate the fluorescence intensity of the tissue spectra. The spectral baseline was corrected to zero by subtracting a constant background (dark current) which was measured along with each spectrum.

Data were reduced by computing an average spectrum and a standard deviation from the 9 to 15 corrected spectra obtained from each site. The average spectrum per site was used in all additional data manipulations. These average per site spectra were grouped according to histologic categories as follows: normal, adenoma, and hyperplastic polyp. For each histologic category, the average and standard deviation spectra were calculated. The results were compared using a one-sided Student's t-test and probability values of less than 0.05 were considered significant.

RESULTS

LIF spectra were obtained in vivo and analyzed from 31 adenomas, 4 hyperplastic polyps, and 32 histologically normal areas from 20 patients. The adenomas ranged from 2 to 11 mm (average, 5 mm) while the hyperplastic polyps measured 2 to 5 mm (average, 3 mm). Twenty-three of the adenomas were classified as tubular while two were villous, and six were tubulovillous. The laser caused no tissue damage that could be detected at the light microscopic level.

Figures 2 to 4 illustrate typical average LIF spectra ± SD for spectra obtained from a representative normal colon, adenoma, and hyperplastic polyp. These data emphasize that the LIF spectra obtained in vivo were reproducible with standard deviations of less than 20%. Superficially, the spectra of normal colon, adenoma, and hyperplastic polyp appeared to have a similar line shape but closer inspection demonstrated differences, especially between the spectra obtained from normal colon and adenoma. Figure 5 illustrates the average of all adenoma spectra superimposed upon the average of all normal spectra. The spectra showed differences in two areas. The fluorescence intensity at 460 nm was approximately four times greater in normal mucosa as compared with adenoma. In addition, the fluorescence intensity at wave lengths greater than 650 nm was consistently greater in adenoma when compared with normal. These differences are quantitatively illustrated in Figure 6 which shows the calculated ratio of the average adenoma spectrum to normal. At wave lengths less than 560 nm, the ratio of fluorescence intensities of adenoma to normal was constant (flat line) and less than one. This indicated that there were no differences in the line shape of the spectra, but the values of fluorescence intensities dif-

Figure 2. A, Typical laser-induced fluorescence spectra collected from a representative specimen of normal colonic mucosa. Note that it is possible to identify individual placements of the probe on the tissue, but that spectral line shape and fluorescence intensity were reproducible. B, Average spectrum ± SD of the individual fluorescence spectra for the normal colonic mucosa shown in A, illustrating the consistency of spectra obtained from a given specimen.

VOLUME 36, NO. 2, 1990

107
than 560 nm, the spectra of adenoma and normal mucosa differed, with the differences maximal near 660 nm. As this wave length corresponded to a peak in fluorescence of adenoma, it was chosen as a region for further study.

There were slight differences in the spectral line shapes in the subcategories of adenomas (tubular adenoma, tubulovillous adenoma, and villous adenoma). The fluorescence line shape of the two villous adenoma

![Graph](image1)

**Figure 3.** Typical average spectrum ± SD for a representative colonic adenoma.

![Graph](image2)

**Figure 4.** Typical average spectrum ± SD for a representative hyperplastic polyp.

At wave lengths greater than 560 nm, the line shape of the spectra differed and the relatively increased fluorescence of adenomas manifested itself as an upward slope in this graph (Fig. 6). Analysis of these data suggested that for diagnostic purposes we could simplify our analysis to a study of the fluorescence intensities at two wave lengths, 460 and 660 nm. As Figure 6 indicates, below 560 nm, the line shape of the spectra of adenomas and normal were similar and the ratio of fluorescence intensities constant and less than one. This indicated that similar diagnostic information could be obtained at any wave length in this region. Experimentally, fluorescence intensity information could be measured most easily at 460 nm, as this corresponds to the peak fluorescence intensity for both adenoma and normal mucosa and there is less noise in the measurement of fluorescence in regions of high fluorescence intensity. At wave lengths greater than 560 nm, the spectra of adenoma and normal mucosa differed, with the differences maximal near 660 nm. As this wave length corresponded to a peak in fluorescence of adenoma, it was chosen as a region for further study.

There were slight differences in the spectral line shapes in the subcategories of adenomas (tubular adenoma, tubulovillous adenoma, and villous adenoma). The fluorescence line shape of the two villous adenomas

![Graph](image3)

**Figure 5.** Average spectrum of all adenomas superimposed upon the average spectrum of all normal colonic mucosal specimens. Note that the fluorescence intensity at 460 nm is approximately four times greater in normal mucosa. Also, fluorescence intensities at wave lengths greater than 660 nm are consistently greater in adenoma.

![Graph](image4)

**Figure 6.** Ratio spectrum produced by dividing the average spectrum of all adenomas by the average spectrum of all normal colonic mucosal specimens. The flat region of wave lengths less than 560 nm indicates that similar diagnostic information is contained throughout this region of the spectrum. Note the increasing slope at longer wave lengths, illustrating the greater fluorescence intensity of adenomas in this region.
was examined differed from the tubular adenomas in that the 620- and 680-nm subsidiary peaks were less distinct. However, there were no significant differences between the average fluorescence intensities in the subcategories of adenomas at 460 nm or 680 nm. This could be due to the small number of measurements in some of the categories.

Figure 7 demonstrates the average fluorescence spectrum of all hyperplastic polyps superimposed on normal and adenoma spectra. The hyperplastic polyp fluorescence intensity at 460 nm lies intermediate between those of adenoma and normal mucosa and closely approximates that of normal at 680 nm.

Table 1 lists the mean fluorescence intensities at 460 and 680 nm for all samples in each histologic category. The fluorescence intensities of hyperplastic polyp and adenoma were statistically significantly different from that of normal mucosa at 460 nm. At 680 nm the fluorescence intensity of hyperplastic polyps was similar to that of normal mucosa while the fluorescence intensity of adenoma was again statistically significantly different. A single two-dimensional scatter plot of the average fluorescence intensity at 460 nm versus 680 nm for each specimen is shown in Figure 8. The scatter plot was divided into two regions corresponding to adenoma and nonadenomatous tissues (normal colon and hyperplastic polyp) using a straight line decision surface chosen to minimize the number of misclassified samples. This decision surface correctly classified 97% of the 67 samples as adenoma or nonadenoma. One normal sample and one hyperplastic polyp were misclassified as adenoma. No adenomas were misclassified. This algorithm retrospectively diagnosed adenoma with a sensitivity of 100% and a specificity of 97%. The predictive value of a positive test for adenoma was 94%.

**DISCUSSION**

Several studies have emphasized that adenomas cannot be reliably distinguished from nonadenoma-

![Figure 7. Average fluorescence spectrum of all hyperplastic polyps superimposed on those of normal and adenoma shown in Figure 5. The hyperplastic polyp fluorescence intensity at 460 nm lies intermediate between the fluorescence intensities of adenoma and normal. At wavelengths greater than 650 nm, the fluorescence intensity of hyperplastic polyps closely approximates that of normal.](image)

![Figure 8. Two-dimensional scatter plot of the fluorescence intensity at 460 nm versus that at 650 nm for all 67 samples. The straight line represents the decision surface which minimizes the number of samples misclassified. One hyperplastic polyp and one normal mucosal specimen were misclassified as adenoma. Using this decision surface, adenoma could be diagnosed with a sensitivity, specificity, and predictive value of 100%, 97%, and 94%, respectively.](image)

**Table 1.**

Average fluorescence intensities ± SD at 460 nm and 680 nm for all histologic categories

<table>
<thead>
<tr>
<th>Histologic category</th>
<th>Average intensity at 460 nm</th>
<th>p*</th>
<th>Average intensity at 680 nm</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.15 ± 0.43</td>
<td></td>
<td>0.037 ± 0.023</td>
<td></td>
</tr>
<tr>
<td>Hyperplastic polyp</td>
<td>0.54 ± 0.16</td>
<td>0.023</td>
<td>6.00 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Adenoma</td>
<td>0.34 ± 0.12</td>
<td>0.001 (versus normal)</td>
<td>0.06 ± 0.05</td>
<td>0.004 (versus normal)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.006 (versus hyperplastic polyp)</td>
<td></td>
<td>NS (versus hyperplastic polyp)</td>
</tr>
</tbody>
</table>

*p Probability values are given for a one-sided Student's t test.
tous mucosa using macroscopic evaluation through the endoscope, especially when dealing with small lesions. The accuracy rate for diagnosis based on gross observation alone generally approximates 76%. As a result, microscopic analysis is necessary and this requires biopsy. Biopsy is time consuming, is associated with some risk to the patient, and leads to additional expense. An alternative approach to endoscopic diagnosis by biopsy may be desirable.

Our results, although preliminary in nature, suggest that LIF spectra can be used in the recognition and differential diagnosis of mucosal abnormalities at endoscopy. Both our in vitro spectral results and our in vivo work demonstrated that the diagnosis of adenomas could be made using LIF spectroscopy with a high degree of accuracy. The in vivo LIF spectroscopy line shapes were, in general, similar to our in vitro observations, except for a 440-nm peak that was observed in some of the adenomas studied in vitro that was not clearly identified in adenomas studied in vivo. We have no explanation for this difference but it is currently under active investigation. Our in vivo LIF spectroscopy results indicated that adenomas could be distinguished from nonadenomatous tissue in approximately 97% of cases. Our results obtained in vivo are comparable to those of Kapadia et al. who studied colonic tissues in vitro and reported an overall accuracy of classification of histologic tissue type of 92% using the excitation wave length of the helium-cadmium laser (325 nm). Since this was presented only in abstract form, spectra were not illustrated and there was no evidence that these investigators determined optimal excitation wavelengths. These authors used a mathematical manipulation of fluorescence emission intensities at several wavelengths (550, 366, 383, and 398 nm) that was not detailed in the abstract in order to discriminate tissue types.

Implications for LIF spectroscopy diagnostic systems are significant. Already, investigators have demonstrated differences in LIF spectra in normal and diseased tissues obtained from human breast, lung, and arterial wall that can be used as a basis for diagnosis. Yaksh et al. have also reported use of LIF spectra in the diagnosis and treatment of colonic adenocarcinomas. This study was done in vitro with 10 resection specimens and described spectral differences between normal mucosa and invasive carcinoma. Invasive carcinoma is usually accompanied by marked collagen deposition (tumor desmoplasia) and since collagen is strongly autofluorescent, the difference in spectra observed by this group could be the result of host response to neoplasm rather than changes inherent to the neoplastic cells themselves.

A fluorescence spectroscopy diagnostic system capable of detecting adenoma (dysplastic/neoplastic transformation) could be of immense practical importance. Currently, no noninvasive technique is available to detect adenoma or other premalignant conditions (dysplasia) in the gastrointestinal tract. The ability to distinguish adenoma from hyperplastic and normal mucosa would save considerable time during colonoscopy as well as decrease the risk and cost of the procedure itself. More importantly, however, the alterations in cellular constituents responsible for the fluorescence spectroscopy line shape differences seen in adenomas studied in vivo may not be unique to the dysplasia associated with colonic adenomas. Premalignant changes (dysplasia) associated with Barrett’s esophagus and mucosal ulcerative colitis frequently are histologically similar to adenomas. If the same abnormalities responsible for the fluorescence spectral changes seen in our study are found in even some of the dysplasias associated with Barrett’s esophagus or inflammatory bowel disease, it may be possible to construct a fiber-optic device that can “see” dysplasia (neoplastic transformation) in these conditions. It may then be possible to either diagnose dysplasia by fluorescence spectroscopy alone or, at the very least, to direct biopsies to areas more likely to contain dysplasia.

The exact biochemical and morphologic basis for the spectral differences between normal, adenoma, and hyperplastic polyp are currently unknown but are under investigation using macroscopic fluorescence data, fluorescence microscopy. The fact that we were able to use fluorescence spectroscopy to distinguish adenomas from most hyperplastic polyps suggests that the differences in fluorescence spectra were not simply the result of the architectural differences between these polyps. A more detailed understanding of the basis of colonic fluorescence will allow us to construct mathematical models of tissue spectra and extract biochemical information. We have demonstrated that these techniques enhance diagnostic ability in other systems.

As our understanding of the diagnostic information contained in these fluorescence spectroscopy grows, we will also incorporate more sophisticated statistical methods of algorithm development. Finally, a double-blinded in vivo colonoscopy clinical trial is currently underway in which fluorescence spectroscopic data are being collected from similar patients in order to prospectively evaluate the diagnostic algorithm presented in this article. The apparent success of the diagnostic algorithm, coupled with the relative ease with which fluorescence spectra can be collected in real time using a standard colonoscope, suggests that fluorescence spectroscopy may soon prove to be a useful method of screening patients for colonic adenomas and other types of colonic dysplasia.

ACKNOWLEDGMENT

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GASTROINTESTINAL ENDOSCOPY
REFERENCES