Confocal laser endomicroscopy

The American Society for Gastrointestinal Endoscopy (ASGE) Technology Committee provides reviews of new or emerging endoscopic technologies that have the potential to have an impact on the practice of GI endoscopy. Evidence-based methodology is used, with a MEDLINE literature search to identify pertinent preclinical and clinical studies on the topic and a MAUDE (U.S. Food and Drug Administration Center for Devices and Radiological Health) database search to identify the reported complications of a given technology. Both are supplemented by accessing the "related articles" feature of PubMed and by scrutinizing pertinent references cited by the identified studies. Controlled clinical trials are emphasized, but in many cases, data from randomized, controlled trials are lacking. In such cases, large case series, preliminary clinical studies, and expert opinions are used. Technical data are gathered from traditional and Web-based publications, proprietary publications, and informal communications with pertinent vendors. For this review, the MEDLINE database was searched through January 2009 using the keywords "confocal," "confocal endoscopy," and "confocal laser endomicroscopy."

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EMERGING TECHNOLOGY

Confocal laser endomicroscopy is a new endoscopic modality developed to obtain very high-resolution images of the mucosal layer of the GI tract. Confocal laser endomicroscopy is based on tissue illumination with a low-power laser with subsequent detection of the fluorescence light reflected from the tissue through a pinhole (Fig. 1).[1] The term *confocal* refers to the alignment of both illumination and collection systems in the same focal plane.[2,3] The laser light is focused at a selected depth in the tissue of interest and reflected light is then refocused onto the detection system by the same lens. Only returning light refocused through the pinhole is detected. The light reflected and scattered at other geometric angles from the illuminated object or refocused out of plane with the pinhole is excluded from detection. This dramatically increases the spatial resolution of confocal endomicroscopy, thus providing an "optical biopsy"—histological examination of the superficial layer of the GI tract.[4,5]

Confocal imaging can be based on tissue reflectance or tissue fluorescence.[6,7] The confocal devices based on tissue reflectance do not require any contrast agents, but available prototypes have had numerous technical problems and relatively low resolution, which significantly compromise in vivo imaging and clinical utility.[6-9]

In contrast, confocal endomicroscopy based on tissue fluorescence uses local and/or intravenous contrast agents and generates high-quality images comparable with traditional histological examination.[5,10] Most clinical studies reported to date used a confocal fluorescence microscope integrated into the distal tip of a conventional upper endoscope (EG-3870CIK; Pentax, Tokyo, Japan) or colonoscope (EC-3870CILK; Pentax). A smaller number of studies used a dedicated confocal miniprobe with laser microscope (Mauna Kea Technologies, Paris, France) inserted through the accessory channel of a traditional endoscope. These instruments have been cleared by the U.S. Food and Drug Administration, and all have different depths of imaging, field of views, and lateral resolutions.

The latest model of Cellvizio confocal miniprobes (Mauna Kea Technologies) created for GI tract applications include CholangioFlex, GastroFlex (ColoFlex), and GastroFlex^{UHD} (ColoFlex^{UHD}). CholangioFlex probes designed for use during ERCP require an endoscope accessory channel of at least 1.2 mm, whereas the other probes, which are designed for use in EGD and colonoscopy, require a channel larger than 2.8 mm. All probes generate dynamic (12 frames per second) images. The depth of imaging for CholangioFlex probes is 40 to 70 μm, 70 to 130 μm for GastroFlex (ColoFlex), and 55 to 65 μm for GastroFlex^{UHD} (ColoFlex^{UHD}). The maximal field of view for CholangioFlex probes is 325 μm, 600 μm for GastroFlex (ColoFlex), and 240 μm for GastroFlex^{UHD} (ColoFlex^{UHD}). The lateral resolution for CholangioFlex

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and for GastroFlex (ColoFlex) probes is 3.5 μm, and the lateral resolution for GastroFlex^{UHD} (ColoFlex^{UHD}) is 1 μm.[11-13] Confocal probes are reusable and expected to last approximately 20 studies.

The confocal microscope integrated into the conventional endoscope collects images at a scan rate of 1.6 frames per second (1024 × 512 pixels) or 0.8 frames per second (1024 × 1024 pixels) with an adjustable depth of scanning ranging from 0 to 250 μm, a field of view of 475 × 475 μm, a lateral resolution of 0.7 μm, and an axial resolution of 7 μm.[14-19]

The fluorescent contrasts for confocal endomicroscopy can be administered intravenously (fluorescein [Pharmalab, Lane Cove, New South Wales, Australia]) or topically (acriflavin [Sigma Pharmaceuticals, Clayton, Victoria, Australia], tetracycline, cresyl violet [AnaSpec, Inc, San Jose, Calif]) through a spraying catheter.[10,20] Intravenously delivered fluorescein distributes throughout the extracellular matrix of the surface epithelium and lamina propria but does not stain cell nuclei.[2] Topically administered acriflavin stains cell nuclei of the surface epithelium but does not penetrate to deeper layers of the GI mucosa.[2] Acriflavin is a mutagenic dye and a potential human carcinogen, which will likely limit its clinical utility.[21,22]

Fluorescein is usually administered within 5 minutes of imaging; however, the dose and timing of contrast administration have not been standardized. After the contrast administration, the tip of the confocal endomicroscope or miniprobe is positioned in gentle contact with the area of interest to obtain high-resolution confocal images. Accumulated images can be saved for postprocedural analysis.

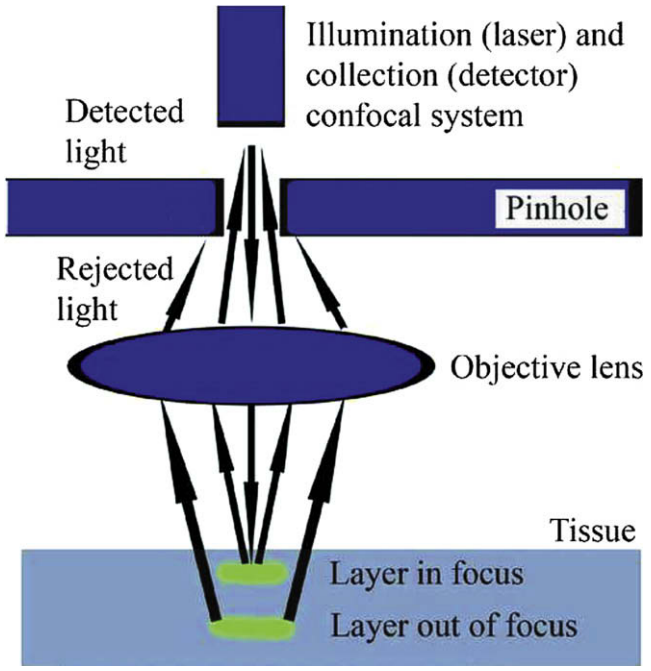

Figure 1. Schematic of confocal endomicroscopy principles.

POTENTIAL APPLICATIONS

Numerous studies have addressed the clinical applications of confocal endomicroscopy. The first prospective human trial evaluated confocal endomicroscopy during screening for colorectal cancer in 69 patients.[16] The presence of neoplastic changes in a polyp was predicted with high accuracy (97.4% sensitivity, 99.4% specificity, 99.2% accuracy).[16]

Another promising approach for the early detection of colonic adenomas, colorectal cancer, and other epithelial malignancies is the development of fluorescein-conjugated peptides that target dysplastic colonic cells. In one study, confocal miniprobes were used to image topically administered peptide in patients undergoing colonoscopy. The fluorescein-conjugated peptide helped to identify dysplastic colonocytes with 81% sensitivity and 82% specificity.[23]

In a large randomized, controlled study of 161 patients with long-term ulcerative colitis referred for a surveillance colonoscopy,[19] chromoscopy with supplemental confocal endomicroscopy resulted in a 4.75-fold higher yield of neoplastic lesions relative to conventional colonoscopy ($P = .005$). In addition, 50% fewer biopsy specimens ($P = .008$) were required.[19] Endomicroscopy was also highly accurate in predicting neoplasia (94.7% sensitivity, 98.3% specificity, 97.8% accuracy).[19]

In patients with Barrett's esophagus, confocal endomicroscopy can distinguish between different types of epithelial cells and detect dysplasia and neoplasia.[24-26] Surveillance endoscopy in 63 patients with Barrett's esophagus provided in vivo histology of the mucosal layer and was able to diagnose Barrett's epithelium and Barrett's-associated neoplastic changes with 98.1% and 92.9% sensitivity and 94.1% and 98.4% specificity, respectively (96.8% and 97.4% accuracy, respectively).[25]

Confocal endomicroscopy in the stomach allows direct in vivo identification of *Helicobacter pylori* infection and good visualization of normal and pathologic gastric pit patterns, making it a potentially useful tool for diagnosis of gastric cancer and precancerous conditions.[3,14,27]

In patients with suspected celiac disease, confocal endomicroscopy can demonstrate villous atrophy and an increased number of intraepithelial lymphocytes, enabling immediate in vivo diagnosis of celiac disease.[3,28,29]

Confocal endomicroscopy can also be helpful for the diagnosis of microscopic colitis. In patients with collagenous colitis, it allows in vivo direct visualization of collagenous bands under the epithelial layer of the colon, and in patients with lymphocytic colitis, it can demonstrate crypt distortion and an increased distance between the colonic

crypts caused by increased mononuclear infiltration in the lamina propria.[30,31]

AREAS FOR FUTURE RESEARCH

Currently available devices for confocal endomicroscopy have a very narrow field of view and allow only visualization of the superficial mucosal layer of the GI tract. Further technological developments are needed to enlarge the field of view, which will facilitate the use of confocal endomicroscopy for cancer screening and surveillance. Increased depth of penetration is also needed to assess depth of invasion during cancer staging.[7,18]

Development of organ- and tissue-specific contrast agents will further expand the indications for confocal endomicroscopy, which can potentially be used to assess extraluminal (eg, biliary, pancreatic, intraperitoneal) pathology.[1]

Confocal endomicroscopy is an examiner-dependent technology. Interobserver and intraobserver variability of this technique has not been adequately studied.[7] Finally, adequate histopathology training is needed for interpretation of confocal endomicroscopy images by gastroenterologists performing this procedure.[7]

The incremental clinical benefit and cost-effectiveness of this imaging modality relative to conventional histopathology examination require further study.

SUMMARY

In recent years, confocal laser endomicroscopy rapidly moved from the bench to the bedside. It is being analyzed as a potentially valuable addition to conventional endoscopy as a means of in vivo optical biopsy enabling real-time histological examination of the superficial layer of the GI tract. How this will affect the practice of screening, surveillance, and early diagnosis of benign, premalignant, and malignant lesions of the GI tract requires further study.

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