High-resolution and optical molecular imaging for the early detection of colonic neoplasia CME

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Colorectal cancer screening is a recommended and widely accepted component of preventive medicine for high-risk patients and asymptomatic adults in the United States.[1] Effective screening depends on detecting lesions that are amenable to curative treatment. Although endoscopic surveillance has decreased the incidence of colon cancer[2] and provided long-term risk reduction,[3] there is considerable room for improvement. Adenoma miss rates remain at approximately 22% (range 15%-32%),[4] with flat and depressed lesions frequently overlooked. Moreover, the specificity of white-light imaging for distinguishing neoplastic from non-neoplastic lesions remains poor, relying on visible mucosal changes.[5] As a result, benign and/or hyperplastic lesions are often removed, increasing both cost and risk.

Over the past decade, multiple wide-field technologies have been developed with the goal of highlighting suspicious mucosa. These modalities, which include narrow-band imaging, digital I-scan, Fujinon Intelligent Color Enhancement system, and autofluorescence imaging are designed to serve as red-flag techniques, theoretically enhancing the macroscopic view of the colon and the diagnostic accuracy of standard colonoscopy. There are, however, no large randomized trials showing an advantage of these modalities over high-definition white-light endoscopy. Moreover, to more accurately determine whether a polyp is hyperplastic or adenomatous, technologies with a higher spatial resolution are required to increase specificity. By combining these technologies with targeted or molecule-specific contrast agents, an even more precise characterization of a lesion's neoplastic potential is possible. This "combination strategy" offers the potential to better identify and characterize lesions at the point of care. Such an approach may enhance detection and treatment strategies by preventing the unnecessary removal of benign lesions and facilitating margin determination during endoscopic therapy. Optical molecular imaging can also provide critical prognostic and therapeutic information such as the presence or absence of a biomarker that can be used to guide drug therapy.

This review provides an overview of the currently available optical biopsy technologies including confocal laser endomicroscopy (CLE), endocytoscopy (EC), and optical coherence tomography (OCT) (Table 1). We reviewed the existing primary data evaluating these technologies used in colon cancer screening and included the pertinent literature. In addition, we review several emerging trends in optical imaging, including the development of lower cost microendoscopic devices and targeted contrast agents. These exciting developments offer the opportunity to enhance the accuracy and efficiency of current screening and the ability to guide decision making in real time.

CONFOCAL LASER ENDOMICROSCOPY

Basic principles

Confocal laser microscopy relies on the excitation of a fluorescent molecule and detection of its emission at a specific axial depth within a given sample. In confocal, a low-power laser is focused on a single point on a fluorescent sample and its emission at that point is recorded, creating 1 pixel of an image. The laser sequentially scans specific points on the specimen in a raster pattern to map out a full picture. Emitted light is passed through a pinhole, eliminating out-of-focus light and creating detailed, high-resolution, subcellular images. Combining this technology with endoscopy allows for contrast-enhanced tissue to be examined on a microscopic scale during colonoscopy, thus offering an in vivo diagnosis or optical biopsy. There are 2 confocal imaging systems commercially available: one that uses a miniaturized scanner within the tip of a conventional endoscope (Pentax/Hoya, Tokyo, Japan), and a probe-based device that is passed through the en-

Abbreviations: CLE, confocal laser endomicroscopy; EC, endocytoscopy; eCLE, embedded confocal laser endomicroscopy; EGFR, epidermal growth factor receptor; LIF, laser-induced fluorescence imaging; OCT, optical coherence tomography; 2-NBDG, 2-(N-(7-nitrobenz-2-ox-1,3-diazol-4-yl)amino)-2-deoxyglucose; pCLE, probe-based confocal laser endomicroscopy; VEGF, vascular endothelial growth factor.

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TABLE 1. Characteristics of currently available high-resolution imaging systems

Imaging system	Contrast agent	Spatial resolution	Sample of published data			
			Trial	No. of patients	No. of lesions	Results
CLE (Pentax)	Fluorescein or acriflavine	0.7 μm	Kiesslich et al,[10] 2004	42	134	99.2% accuracy, 97.4% sensitivity, 99.4% specificity
			Sanduleanu et al,[11] 2010	72	116	95.7% accuracy, 97.3% sensitivity, 92.8% specificity
CLE (Miniprobe)	Fluorescein or acriflavine	1-3.5 μm	Meining et al,[12] 2007	47	36	91.7% accuracy, 92.3% sensitivity, 91.3% specificity
Endocytoscopy	Methylene blue	1.7-4 μm	Sasajima et al,[15] 2006	60	75	93.3% Accuracy

CLE, Confocal laser endomicroscopy.

doscope's working channel (Mauna Kea Technologies, Paris, France).

Equipment and technique

The Pentax confocal laser endomicroscope incorporates an embedded miniaturized laser scanner into the distal tip of the colonoscope, along with a flexibly connected solid-state laser (eCLE). The solid-state laser delivers blue laser light (488 nm) via a single optical fiber. Within the laser scanner, there is a lens system that focuses returning light onto the end of the optical fiber (acting as the confocal pinhole), thus eliminating light from other imaging planes. For the probe-based CLE system (pCLE), a miniprobe consisting of a bundle of optical fibers is passed through the accessory channel of a standard colonoscope (with a 2.8-mm channel) and connected to a more conventional laser scanning unit and detector. The scanning unit sequentially scans each fiber to collect the pixels of the image.[6]

Both of these systems can achieve similar fields of view depending on the specific fiberoptic probe used (240-600 μm for pCLE and 475 μm for eCLE). However, compared with eCLE, pCLE has slightly less lateral resolution (0.7 μm vs 1.0-3.5 μm) and significantly less axial resolution (<1 μm vs 15 μm). eCLE also has the advantage of producing images of structures from 0 to 250 μm in depth, whereas with the miniprobe, the depth of images ranges from 0 to 120 μm, depending on which probe is used. Because of these specifications, however, pCLE is able to achieve a much higher rate of image acquisition than eCLE and is able to create a true "video mosaic" of images. This allows visualization of a larger portion of the mucosa.[7]

Contrast agents

Both CLE systems require exogenous fluorescent contrast agents to allow for high-resolution images. These agents can be applied topically or intravenously, once a suspicious area of the mucosa is identified during a standard colonoscopy.

Fluorescein is the most commonly applied contrast agent used in endomicroscopy and has been extensively used in retinal angiography. With administration of intravenous fluorescein, blood vessels, intracellular spaces, and lamina propria are all highlighted, whereas nuclei and mucin remain unstained. This provides cellular and subcellular details and connective tissue and blood vessel architecture.[8] The rate of complications is low and generally limited to transient yellowing of the skin, eyes, and urine. Nausea and vomiting can occur, and anaphylaxis or allergic reactions are rare.

An alternative, topical contrast agent is acriflavine hydrochloride. Acriflavine binds to nucleic acids, staining nuclei and cytoplasm to a depth of 100 μm. Acriflavine typically does not highlight the microvasculature or connective tissue in deeper layers of the mucosa.

Interpretation

Several features on confocal images can differentiate normal from dysplastic or neoplastic lesions in the colon. In normal mucosa and hyperplastic polyps, nuclei are rarely visualized. In addition, the epithelial components display a cohesive architecture, and the microvasculature appears in a normal honeycomb pattern. Red blood cells are also visualized and appear as moving black dots.[9] Conversely, in neoplastic lesions, denser and enlarged nuclei may be visualized, the epithelial architecture is disrupted, and the vasculature displays irregularity as well as leakage of fluorescein, which represents neoangiogenesis (Fig. 1).

Limitations

There are several limitations to this technique. Because of the extremely small field of view (<700 μm), this technique is time-consuming. In addition, the cost of these systems is more than $100,000, and there is a significant