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Endomicroscopy, endocytoscopy, and autofluorescence for polyp characterization ☆

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ABSTRACT

This mini review deals with autofluorescence and cellular imaging using endomicroscopy or endocytoscopy during colonoscopy. Autofluorescence can be used to detect and characterize colorectal lesions whereas endomicroscopy and endocytoscopy are techniques to characterize colonic polyps based on cellular and subcellular patterns.

1. Introduction

Colon cancer is the second most common cancer both in women and men [1]. Colonoscopy is the gold standard for screening and surveillance of colon cancer. Multiple new endoscopic technologies are available which aim to improve colonoscopic diagnosis [1].

This review focuses on autofluorescence (AF) and cellular imaging, which facilitate polyp characterization. Cellular imaging can be achieved using endocytoscopy and endomicroscopy. Endocytoscopy provides super magnification of the colonic surface whereas endomicroscopy devices can analyze the surface layer or the entire thickness of the mucosa at subcellular resolution.

Confocal laser endomicroscopy (CLE) is of special interest because it provides not only cellular imaging but also molecular imaging.

AF seems to be of low clinical relevance whereas endomicroscopy shows very promising results for future use and clinical research. At the present time, endomicroscopy is not ready for daily clinical use because of time and cost constraints. This technology will be more available when new, easier to use, and cheaper endomicroscopic systems become available.

2. Technical aspects of CLE

CLE is based on tissue illumination with a low-power laser after topical (ie, cresyl violet and acriflavine hydrochloride) or intravenous (fluorescein sodium) application of fluorescence agents. Fluorescein sodium in a dilution of 10% is the most commonly used agent. After intravenous injection fluorescein is dispersed into and highlights the extracellular matrix. Adverse events are rare; 1 multicenter study reported transient hypotension without shock (0.5%), nausea (0.39%), injection site erythema (0.35%), self-limited diffuse rash (0.04%), and mild epigastric pain (0.09%) [2]. Fluorescein sodium can be combined with topical application of acriflavine hydrochloride to visualize the cell nucleus. This dye agent allows for a detailed analysis of the nucleus-to-cytoplasm ratio for the diagnosis and grading of intraepithelial neoplasia. As acriflavine accumulates in the nuclei, concerns have been raised regarding a potential mutagenic risk [3]. Alternatively, cresyl violet can be applied. By cytoplasmic enrichment of cresyl violet, nuclear morphology can be "negatively" visualized [4].

Two food and drug administration-approved CLE devices are available for use in clinical practice. One is integrated into the distal tip of a standard high-resolution video gastroscope or colonoscope (iCLE; Pentax Medical, Tokyo, Japan) [5] and the other is probe-based, capable of passage through the working channel of a standard endoscope (pCLE; Cellvizio, Mauna Kea Technologies, Paris, France) [6].

Both systems use an incident 488-nm wavelength laser system. iCLE collects images at a manually adjustable scan rate of

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Table 1
Technical aspects of integrated (iCLE) and probe-based (pCLE) confocal laser endomicroscopy.

	iCLE	pCLE
Outer diameter (mm)	12.8	1.0; 2.7; 2.6*
Length (cm)	120; 180	400; 300*
Field of view (μm)	475 × 475	320; 240; 600*
Resolution (μm)	0.7	3.5; 1.0*
Magnification	×1000	×1000
Imaging plane depth (μm)	0-250 (dynamic)	40-70; 55-65; 70-130 (fixed)*

*Dependent on various probes.

1.6 frames per second with a maximum resolution of 1024 × 1024 pixels (1 megapixel). By pushing a button on the handle of the endoscope, one can dynamically adjust the scanning depth (ranging from 0-250 μm) and the laser power (ranging from 0-1000 μW). For pCLE, different probes for various indications are available. pCLE devices use a dynamic laser power and a fixed imaging plane depth. Confocal images are streamed at a frame rate of 12 frames per second thereby obtaining real-time video of the intestinal mucosa. A special computer algorithm ("mosaicing") allows reconstruction of single video frames either in real time or postprocessing, with an increased field of view of up to 4 × 2 mm. Table 1 provides an overview of main technical aspects of both CLE devices (Figures 1-3).

3. Technical aspects of endocytoscopy

Endocytoscopy (Olympus, Tokyo, Japan) is based on the principle of contact light microscopy, thereby only permitting visualization of the superficial mucosal layer [7]. Images are displayed on a monitor at 30 frames per second. The endocytoscope is either integrated into a handheld device, which can be advanced through the working channel of an endoscope (called pEC), or integrated into a high-resolution endoscope (called iEC). Endocytoscopy requires mucolysis with *N-acetylcysteine* and prestaining of the mucosa with absorptive staining agents like methylene blue, toluidine blue, or cresyl violet at high concentration. Usually, the dyes are sprayed onto the mucosa by using

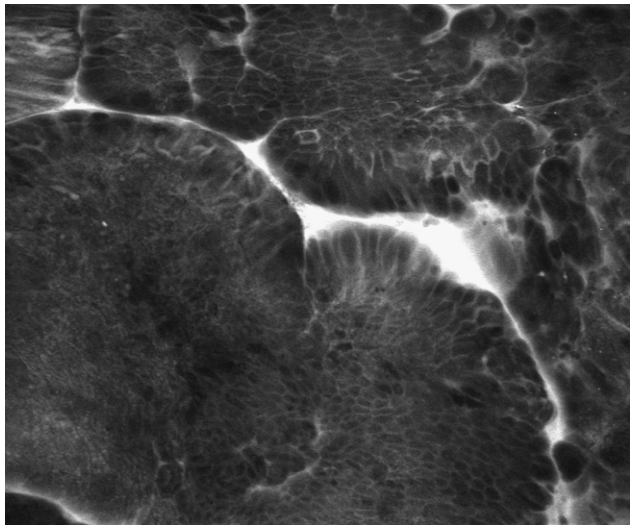

Fig. 2. Confocal image (iCLE) of sporadic colonic tubular adenoma with low-grade intraepithelial neoplasia. Adenomatous tissue shows depletion of goblet cells and columnar cell alignment.

standard spraying catheters. After an appropriate time of exposure of the dye (about 60 seconds), repeat washing of the mucosa is mandatory to remove excess contrast dye before endocytoscopic (EC) imaging can be started. Repeat staining is mainly necessary when using absorptive contrast agents. Table 2 provides an overview of the different endocytoscopy systems which are currently available. It is noteworthy that a clear distal cap and mild suction should be used to stabilize the endoscope while obtaining high-magnification imaging of the mucosa. Interpretation of EC images is based on architectural details (eg, epithelial structure), cellular features (eg, size and arrangement of cells), and vascular pattern morphology (eg, size, leakage, and tortuosity). Additionally, endocytoscopy allows for the assessment of cytologic features, such as the density of cells, size and shape of nuclei, and nucleus-to-cytoplasm ratio. Until now, endocytoscopy is not commercially available in Europe or the United States, and has been evaluated by Japanese colleagues for colorectal polyps and early cancer [8].

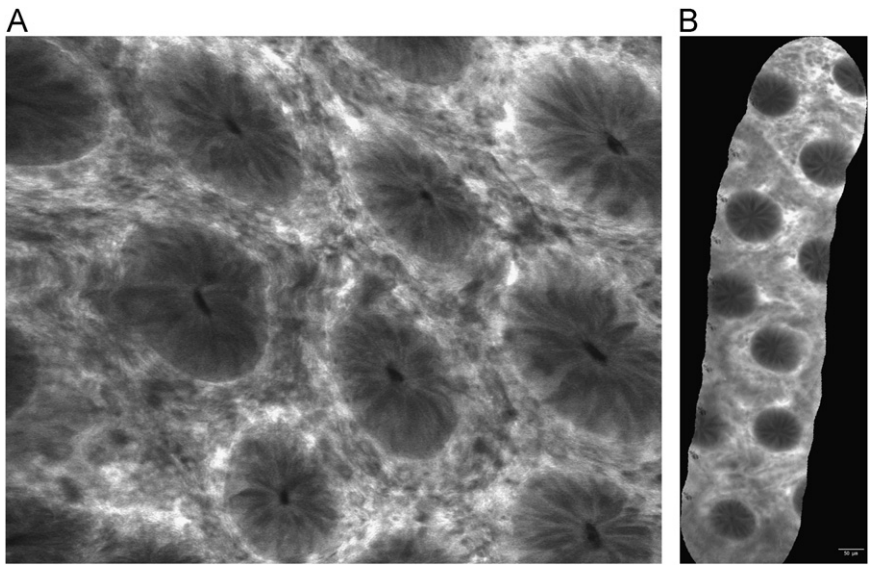

Fig. 1. Normal appearance of colonic mucosa using confocal laser endomicroscopy (CLE) (A) was imaged using iCLE (integrated into distal tip of endoscope) and (B) was imaged with pCLE (probe-based) using mosaicing that allows reconstruction of single video frames with an increased field of view.