Konfokalna endomikroskopija: mikroskopija ob endoskopiji

Confocal endomicroscopy: Microscopy in endoscopy

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Izvleček

Konfokalna endomikroskopija je nedavno razvita preiskovalna metoda, ki omogoča podpovršinsko histološko diagnostiko živega tkiva, tako na celični kot tudi podcelični ravni. Prinaša možnost neposrednega histopatološkega pregleda tkiva med endoskopsko preiskavo prebavil in prepoznavo neoplastičnih ali vnetnih sprememb sluznice. Raziskave so potrdile, da je konfokalna endomikroskopija uporabna pri presejalni koloskopiji, ulceroznem kolitisu, diagnostiki Barrettovega požiralnika in raka želodca. Raba pri živalskih modelih bolezni omogoča funkcijsko slikanje raka, perfuzijskih vzorcev pri malignih in vnetnih boleznih in omogoča visokoločljivostni morfološki prikaz živega tkiva. Številne raziskave so usmerjene v razvoj in uporabo molekulskih označevalcev, uporabnih pri imunohistokemičnem označevanju tkiva *in vivo*. Konfokalna endomikroskopija se uspešno razvija v uporabno metodo, namenjeno hitri in natančni prepoznavi vnetnih in neoplastičnih bolezni na mikroskopski ravni že med endoskopsko preiskavo.

Abstract

Confocal endomicroscopy is a novel technology, which allows subsurface histological diagnosis at a cellular and subcellular level *in vivo*. It thereby provides instantaneous histopathology during ongoing upper and lower endoscopy. This allows immediate diagnosis of neoplastic and inflammatory lesions of the intestinal mucosa. Studies have demonstrated the power of confocal endomicroscopy in screening and surveillance colonoscopy, ulcerative colitis, Barrett's esophagus, and gastric cancer. In animal models of human diseases, the same technology has provided molecular imaging of cancer, functional imaging of altered perfusion in malignant and inflammatory disease and high resolution in vivo morphological diagnosis. Fields of ongoing research are the development of molecular markers for in vivo immunohistochemistry and the application of confocal microscopy to intraabdominal organs in humans. Confocal endomicroscopy is evolving as a novel technique for rapid intravital diagnosis of gastrointestinal inflammatory neoplastic diseases at the microscopic level and bears the potential for molecular imaging in humans in the future.

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INTRODUCTION

In confocal laser endomicroscopy, a low-power laser is used to focus onto a single point within the tissue. Light, emanating from this point, is focussed through a pinhole to a detector while the laser raster scans the transverse imaging plane. Light from outside this focally illuminated point or plane is geometrically rejected and does therefore not blur the resultant image. This allows the detection of surface and even subsurface microscopic details.

For confocal endomicroscopy, a miniaturized scanning head has been integrated into the distal end of a modified CCD-videoendoscope (Fig. 1, Pentax EC-3870CIFK, Pentax, Tokyo, Japan). A solid state laser delivers a wavelength of 488 nm for fluorophore excitation. Light emission from the tissue is detected at 505 to 555 nm. The resultant image corresponds to a transverse optical section through the tissue. The optical slice thickness of this section is 7 µm, and the lateral and axial resolution 0.7 µm. The field of view is 475 x 475 µm. Imaging plane depth with blue laser illumination can be manually varied from mucosal surface down to 250 µm. Actuation of the imaging plane depth along the range of the z-axis is controlled using two remote control buttons on the handpiece of the endoscope. Laser power output at the tissue surface can be adjusted during ongoing endoscopy from 0 to 1000 µW to achieve appropriate tissue contrast. Serial image frames are collected on a screen separate from the simultaneously displayed videoendoscopic image at a scan rate of 0.8 frames/s at 1,024 x 1,024 pixels or 1.6 frames/s at 1,024 x 512 pixels, approximating a 1000-fold magnification on a 19-inch screen. Confocal images can be captured with the help of a foot pedal and are digitally stored as gray-scale images. The confocal endomicroscope (Fig. 1) can be handled similar to a conventional endoscope. When the confocal imaging window is brought in close contact with the mucosa, a microscopic image is displayed on a separate screen simultaneous with

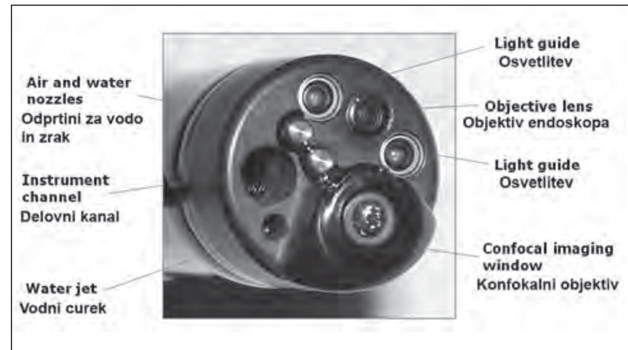

Figure 1. In the confocal endomicroscope, a miniaturized scanning head has been intregrated into the distal tip of an otherwise conventional coloscope. The confocal imaging window is slightly protruding from the tip and can be targeted onto a lesion under videoendoscopic control. Imaging depth is controlled using two additional buttons on the handpiece (FOV 475 x 475 µm)

the videoendoscopic image after the injection of 5 ml fluorescein 10% as a fluorescent agent. Some indications for confocal endomicroscopy are highlighted in the following from a wide variety of clinical entities that have been studied since the launch and CE cerification of confocal endomicroscopy in 2004.

USE OF CONFOCAL ENDOMICROSCOPY IN UPPER GASTROINTESTINAL ENDOSCOPY

In the esophagus, confocal endomicroscopy was prospectively evaluated for the diagnosis of specialized intestinal metaplasia and Barrett's associated intraepithelial neoplasia (IN) in 63 patients with long-lasting reflux symptoms, previously diagnosed Barrett's esophagus or scheduled endoscopic therapy for Barrett's associated neoplasias (1). Barrett's epithelium was diagnosed *in vivo* by the presence of villiform or glandular epithelium, the microscopic diagnosis of intrapapillary capillary loops and by the presence of goblet cells, which are pathognomonic for Barrett's epithelium and easily recognized by their dark staining mucin inclusion. With conventional histology from targeted specimens serving as the gold standard, prediction of specialized intestinal epithelium was possible with an accuracy of 96.8%

by confocal endomicroscopy. In Barrett's associated IN, the monolayer of high prismatic cells was visibly disturbed, and in a subset of lesions, the basal membrane was interrupted by black cells that were not contained within the epithelial layer, indicating early stages of infiltration. Accuracy of prediction of IN was 92.9% at 28 imaging sites *in vivo*. Tumor vessels were irregular and tortuous and showed an enhanced leakage of fluorescein into the tissue. Inter- and intraobserver agreement was 0.84 and 0.89, respectively. Recent studies have successfully applied this technique for the in vivo diagnosis of esophageal squamous cell cancer (2), gastric pathologies (3), H. pylori-infection (4) and celiac disease (5).

USE OF CONFOCAL ENDOMICROSCOPY IN LOWER GASTROINTESTINAL ENDOSCOPY

The first studies on confocal endomicroscopy have been performed in colonoscopy (6, 7). High accuracy of 99.2% for the distinction of normal mucosa from regenerative (hyperplastic or inflammatory) changes from neoplastic lesions has been established based on a simple to use confocal classification. This experience has been rapidly broadened to a multitude of centres and indications. Here, both acriflavine and fluorescein have been evaluated for their ability to visualise the colonic and ileal mucosa (6, 7). Since fluorescein allows easy, safe, and rapid microscopic visualisation across the whole range of the imaging depth and gives a good overall impression of the mucosal microarchitecture, it has been found more adequate for routine use in colonoscopy than acriflavine.

In a prospective randomized trial (8), 153 patients with longstanding ulcerative colitis in clinical remission scheduled for surveillance colonoscopy were randomized at a 1 : 1 ratio to either conventional video white light colonoscopy or panchromoendoscopy with methylene blue (0.1%) in combination with confocal imaging. In this setting, chromoendoscopy was used to unmask lesions, and confocal endomicroscopy for immediate microscopic classification using the confocal pattern classification. Endomicroscopic prediction of the dignity of lesions was accurate in 97.8% (8). The time to perform a single complete colonoscopic evaluation was 31 minutes for video endoscopy, and increased to 42 minutes by adding confocal endomicroscopy and chromoendoscopy. Endomicroscopy-guided colonoscopy resulted in an average of 21.2 biopsies per patient, while 42.2 biopsies were necessary in conventional colonoscopy. If only circumscript lesions had been biopsied, the number of biopsies could have been further reduced to 3.9 per patient without reducing the number of IN detected. Potentially as important, the negative predictive value for mucosa with a normal appearance on endomicroscopy not to contain IN was 99.1%. In this study, *in vivo* microscopically targeted biopsies increased the diagnostic yield of surveillance colonoscopy in ulcerative colitis patients. At the same time, this method allows the pathologist to focus on targeted biopsies from suspicious lesions only. This study has been recently confirmed in a follow-up study from Great Britain (9). Here, confocal endomicroscopy and chromoendoscopy have been compared to chromoendoscopy alone. Even in this setting, targeting biopsies in vivo by the use of confocal endomicroscopy has increased the yield of IN by a factor of 2.5.

In a similar approach (10), confocal endomicroscopy was used to differentiate sporadic adenomas in ulcerative colitis from dysplasia-associated lesions or masses (DALM). While sporadic adenomas can be treated by complete endoscopic resection, the presence of DALM with high grade dysplasia constitutes an indication for procto-colectomy. Confocal endomicroscopy was able to identify areas with dysplastic tissue around a lesion in DALM, whereas adenomas were surrounded by normal colonic mucosa. This finding potentially translates into immediate clinical consequences of resection *versus* biopsy and surgery, enabling on-table patient management.

SUMMARY AND PERSPECTIVE

Confocal endomicroscopy is a fascinating new technology that for the first time enables *in vivo* histopathology during ongoing endoscopy. This novel technique relies on fluorescent intravital staining that reveals morphology at a microscopic level. Confocal endomicroscopy requires training, just as any other new endoscopic technique, yet the first studies show encouragingly that endoscopists are able to evaluate virtual optical biopsies by means of simple and easy to apply classification systems, thereby targeting biopsies to the most relevant parts of a lesion and at the same time saving unnecessary random biopsies. Confocal endomicroscopy has been successfully evaluated in randomized trials for a multitude of indications in both upper and lower GI tract.

Current research aims at both broadening the applicability of *in vivo* confocal endomicroscopy.

Our group has recently added molecular and functional imaging to high resolution *in vivo* imaging using a further miniaturized rigid design of the confocal system used for flexible endoscopy (11). Even human colorectal tumours could be specifically labelled and visualized by their molecular properties in a murine model, providing the first evidence that molecular imaging by confocal microscopy can be achieved *in vivo* using a system compatible with application in humans (12).

Taking together the multitude of randomized trials from many different centres around the world and the large and ever enlarging number of indications, confocal endomicroscopy will result in a truly comprehensive morphological and molecular imaging. *In vivo* confocal endomicroscopy therefore not only greatly facilitates the diagnosis of gastrointestinal pathologies already today, but also bears the potential to allow for a unique dynamic look into cellular life, disease and death *in vivo*.

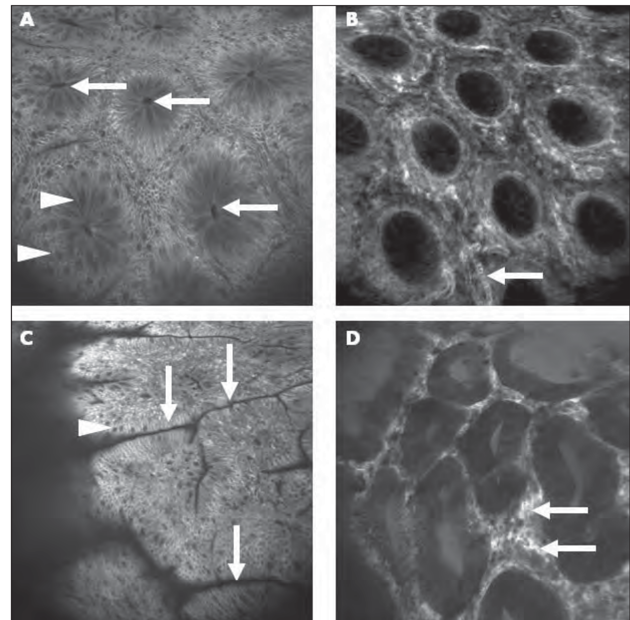

Figure 2. Colonic mucosa: Figure 2A shows normal colonic mucosa with an en-face view onto the crypt openings (arrows). Those are regular, and the crypts hexagonal. Dark inclusions at the luminal side indicate mucin in goblet cells (arrowheads). In deeper sections (Figure 2B), the meshwork of capillaries is visualized. Each crypt is fed and drained by a surrounding vessel, perfused by fluorescent plasma after intravenous injection of fluorescein sodium. Black dots within the vessel lumina correspond to single red blood cells (arrows) and allow a rough estimation of the size of the vessel. In neoplastic tissue (adenoma, Figure 2C), the regular architecture is progressively lost. The surface appears villous, and crypts are elongated (arrows) or fuse laterally, corresponding to type III and IV pit pattern, resp. Goblet cells (arrowheads) are rare. In deeper optical sections (Figure 2D), enhanced leakage of fluorescein is seen (arrows), indicating enhanced vessel permeability (FOV 475 x 475 µm).

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