Application of Confocal Laser Endomicroscopy in Clinical Practice

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Introduction

Confocal laser endomicroscopy (CLE), an adaptation of confocal laser scanning microscopy, uses laser light for excitation and captures laser-induced fluorescence from a defined imaging plane depth, provides high resolution microscopic images at subcellular resolution, and enables deep as well as superficial tissue imaging. However, submucosal infiltration cannot be detected by current wavelengths. Usually, intravenous fluorescein has been used to improve the optical contrast in cellular, subcellular, connective tissue and vessel architecture at high resolution, but does not stain nuclei.¹,²

Many studies on CLE have demonstrated the ability of gastroenterologists to obtain and interpret microscopic images of the gastrointestinal tract during endoscopy. The information from CLE has been successfully used to minimize sampling error by microscopically targeted biopsies and to guide endoscopic interventions. In addition, CLE also can visualize cellular changes in their natural microenvironment free of artefacts, enabling fundamental insights into mechanisms of intestinal diseases in clinical and translational science.¹,²

The CLE must be performed in direct contact with the mucosa. CLE images represent optical sections parallel to the horizontal tissue surface, which is in 90° to conventional histopathologic section. For the interpretation of microscopic images, adequate training is needed in the endoscopic technique and knowledge of mucosal histopathology. Because CLE provides high magnification within a small field of view, CLE can be combined with chroendoendoscopy or HD-WLE (high definition white light endoscopy) to identify suspicious areas.² Currently, two types of CLE are available, the Pentax CLE, integrated into the tip of the conventional endoscope (eCLE), and probe-based CLE (Cellvizio; Mauna Kea Technologies, Paris, France), which can be used through the accessory channel of traditional endoscopes (pCLE).

Colorectal Neoplasia

CLE not only enable prediction of histology, but actual visualization of microscopic tissue details in real time during endoscopy. Therefore, it has resulted in the use of intravital microscopy to actually plan and guide endoscopic interaction.

Initial studies demonstrated the utility of CLE in the evaluation of colonic polyps.³ CLE had 97% sensitivity and 99% specificity, when compared with histology of colonic mucosa including areas with neoplasia.³ This study showed the basic principle that CLE can provide microscopic images during endoscopy and that endoscopists are able to interpret microscopic images on-site. On the basis of a simple-to-use classification, colonic neoplasia could be differentiated from regenerative and normal mucosa with 99% accuracy. In addition, differentiation of the grade of colorectal neoplasia was possible with a combination of systemic fluorescein and topical acriflavine,⁴ and a similar classification system using pCLE has been proposed.⁵

There have been some data on the comparison of CLE with HDWLE and virtual or real chroendoendoscopy. Initially,
Recently, pCLE has been shown to be superior to NBI (narrow-band imaging) in prediction of colonic histology (91% vs 77%). This finding was found in a study comparing pCLE with virtual chromoendoscopy for the detection of residual neoplasia in colonic scars after mucosal resection. However, these promising results have not been reproduced in other studies, showing that pCLE was inferior to both NBI and dye-based chromoendoscopy for the characterization of colonic polyps (72% vs 89%, respectively). These results might be explained in part by difficulties in obtaining good microscopic quality of CLE images, and by increasing quality and experience with new HDWLE. Another study examining the interobserver variability between endoscopists in distinguishing neoplastic from non-neoplastic polyps with pCLE showed a sensitivity of 76% and specificity of 72%, and interobserver agreement ranged from moderate to good. In addition, use of CLE before endoscopic mucosal resection was more accurate at diagnosing neoplasia compared with biopsies, because a larger area of the lesion could be screened microscopically, reducing the risk of sampling error.

Non-neoplastic Colorectal Diseases

1. Inflammatory Bowel Disease

Intravital visualization has been utilized for microscopically guided ‘smart’ biopsies in diseases affecting large mucosal areas, because conventional random biopsies are subject to substantial sampling error. CLE has also been used to characterize suspicious lesions noted during pancolonic chromoendoscopy during surveillance colonoscopy for ulcerative colitis. A study in UC (ulcerative colitis) patients showed that CLE combined with chromoendoscopy increases the detection rate of dysplastic lesions 4.75-fold with an accuracy of 97.8% in comparison to conventional colonoscopy. CLE showed 50% fewer biopsy specimens were required, suggesting usefulness of chromoendoscopy-guided CLE to abrogate the need for random biopsies and improve the clinical management of UC. Other study with longstanding IBD (inflammatory bowel disease), using pCLE in conjunction with HD NBI endoscopy, showed an accuracy of 81% in predicting neoplasia in detected lesions. They also found that pCLE added 30 minutes to the procedure, and good-to-excellent video images of pCLE were only obtained in about two-thirds of the patients. CLE has also been shown in small pilot studies to be useful in detecting inflammatory activity in the colonic mucosa of both Crohn’s disease and UC. In addition, on the relevance of achieving mucosal healing in IBD, CLE will have a role as an excellent tool for microscopic surveillance of inflammation under therapy.

In subcellular level of IBD, CLE could provide additional information. The gut surface is punctuated by gaps after single cell shedding from intestinal epithelial lining, which are usually sealed to keep barrier function intact as a part of normal tissue regeneration. In CLE examination, such gaps were found more frequently in patients with IBD, compared with in healthy individuals. In some IBD patients, these gaps also show an increased transepithelial flow across the epithelium even in the absence of stigmata of an IBD flare in conventional histopathology. In addition, such an impaired barrier function was predictive of about four-fold increased risk of relapse in the following 12 months, suggesting these gaps could be related to bacterial translocation into the mucosa, which is visualized more frequently by eCLE in patients with IBD compared with controls. Furthermore, these gaps have been also found in the healthy duodenum of IBD patients, supporting previous findings of impairments in the epithelial barrier even in the absence of gross or microscopic changes on conventional endoscopy or biopsy.

2. Other intestinal diseases

In further advanced step, CLE could be used for translational studies of epithelial function and molecular imaging due to its unique ability to visualize details at subcellular level in their natural microenvironment, free of the artifacts
induced by biopsy sampling.\textsuperscript{22,23}

Therefore, CLE examination can obtain continuous imaging of microscopic events, and also visualize functional tissue properties such as permeability, perfusion, or cellular shedding that are usually not well addressed by \textit{ex vivo} imaging. For example, CLE imaging of epithelial apoptosis after co-staining with acriflavine could predict graft-versus-host disease after bone marrow transplantation in real time, suggesting immediate therapy for this threatening disease condition.\textsuperscript{24}

Similarly, pCLE could identify an increased gap density in the terminal ileum of patients with IBS, compared to healthy individuals,\textsuperscript{25} indicating increased cell extrusion as a microstructural factor of altered mucosal permeability observed in these IBS patients. Therefore, in the future, CLE might contribute to our understanding of disease pathogenesis by \textit{in vivo} imaging.

\section*{Conclusions}

CLE examination is currently not popular and usually used for clinical and translational research. Currently, due to its limited field of view, CLE examination needs to be incorporated with HD and dye-based or virtual chromoendoscopy to identify suspicious areas that need CLE evaluation. CLE could be an adjunct to get ‘smart’ fewer biopsies instead of obtaining a larger number of untargeted random biopsy samples. In addition, CLE is not just a real time evaluation of histopathology, but also offers a unique microscopic view into changes of epithelial physiology and pathophysiology in the natural microenvironment. Furthermore, CLE might become more interesting as the ideal tool for on-site diagnosis, when resect-and-discard or diagnose-and-leave-in-place strategies are to be applied to clinical practice.\textsuperscript{26}

However, there have been some issues to be solved, such as substantial amount of time to be added to the endoscopy procedure, limited field of view, cost-effectiveness, and low feasibility in acquisition of good-quality CLE videos and images. Additional studies in various diverse clinical settings are needed before it can be applied in routine clinical practice, extending the role of the endoscopist.

\section*{References}

10. Jeon, S. R. et al. Optical biopsies by confocal endomicroscopy prevent additive endoscopic biopsies before endoscopic submucosal dis-


