Advances in confocal laser endomicroscopy for neuro-oncological tumor resection: a mini-review of current literature

By Carl Zeiss Meditec AG, Medical Technology

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Precise identification of tumor areas is crucial in neuro-oncological tumor resection, particularly in the case of infiltrative gliomas.[1]

Conventional neuropathological assessments during neurosurgery are typically based on rapid frozen-section histopathology of small extracted tissue samples using conventional benchtop microscopes. Different staining methods (e.g. hematoxylin and eosin [H&E] staining) or fluorescent dyes are used to provide sufficient image contrast.

A number of neurosurgical studies have indicated a range of benefits of fluorescence-guided microscopy for glial tumor resection, including increased gross total resection rate and progression-free survival.[12] Some studies have even suggested possible increases in overall patient survival.[12] However, fluorescence microscopy does not provide sufficient spatial resolution or sensitivity to effectively visualize the surgical margins of diffuse tumors.

Intraoperative optical-sectioning with handheld probe-based confocal laser endomicroscopy systems provides superior resolution and sensitivity for the detection of infiltrating cells at tumor margins, and can potentially be used to quantify tumor parameters in localized regions of brain tissue during the final stages of tumor resection.[13]

From the viewpoint of neuropathologists, in vivo CLE may offer several benefits as an adjunct to physical histopathological assessment. First, biopsy quality can be optimized through more accurately targeting diseased tissue. Second, imaging can complement physical biopsy investigations by providing information on the architectural context of the tissue, without visual artifacts that are commonly seen in frozen biopsy tissue.[4] Third, this technology can enable real-time in vivo intraoperative neuropathological consultation, providing practical benefits for tumor resection workflow.[1,2,12]

Technology

In CLE a low-power laser is used to selectively visualize the area of interest in a tissue on a specific focal plane, providing an 'optical section'.[3,15] As with a conventional, benchtop confocal microscope, newer systems adapted for surgical endomicroscopy incorporate a pinhole that eliminates light from outside the desired focal plane (background fluorescence), which enables high spatial resolution during 3-dimensional assessments (in X,Y, and Z), as well as the evaluation of tissue architecture at a cellular level.[2,15]

CLE requires the administration of fluorescent contrast agents immediately before the surgical procedure. Intravenous FNa is generally the preferred fluorophore given its 50-year history of clinical use and a well established safety profile.[15,16] FNa only penetrates into the CNS in areas where the blood-brain-barrier is compromised, as in most gliomas.[1,3] Studies have confirmed that pre-operative intravenous FNa administration allows assessment of tissue microstructure (e.g. visualization of tumor cell nuclear and cell dimensions), differentiation of tumor cells from healthy brain tissue, determination of tumor border regions, and visualization of injury to normal brain tissue and brain vasculature.[1,3]

A number of CLE systems are available, with the confocal tip either integrated into an endoscope (e.g. EC3870K by Pentax, Japan) or into a separate probe (e.g. Cellvizio® by Mauna Kea Technologies, France; ZEISS CONVIVO by Carl Zeiss Meditec AG, Germany). Image data from these systems can be evaluated immediately in the OR, or can be streamed for real-time external evaluation to allow remote neuropathologist support.[3,4] Thus, unlike current standard surgical practice, CLE allows real-time *in vivo* imaging directly in the relevant tissue, supplementing or potentially even replacing physical biopsies.

Currently CLE technology is mainly used in intra-gastrointestinal diagnosis and polyp removal during routine colonoscopy.[17] However, experience in neurosurgical applications in the brain is increasing.[1,18]

Animal model studies

Proof-of-concept studies in a range of animal species (mice, rats and pigs) suggest that CLE with real-time neuropathology assessment could increase efficiency in the brain tumor resection workflow, potentially reducing the time required in the OR and associated costs.[1,4,5] In a laboratory assessment of CLE diagnostic accuracy in a mouse glioma model, neuropathologists were able to differentiate tumor from nontumor tissue with a mean accuracy, specificity, and sensitivity of 90%, 86%, and 96%, respectively, and with high overall interobserver agreement.[6]

Preclinical feasibility studies and initial clinical testing suggest that CLE may enable rapid identification of characteristic (diagnostic) histological features of tumor tissue.[3,7,18–20]

Human *ex vivo* and *in vivo* studies

A number of human clinical studies including *ex vivo* and *in vivo* evaluations have assessed the utility of CLE for real-time intraoperative differential diagnosis.

The effectiveness of CLE was evaluated in a study based on ex vivo human brain biopsies and sections from a number of different tumor types that had distinctive as well as complementary reflectance and fluorescence characteristics. Multimodal imaging including CLE allowed neuropathologists to distinguish gliomas from normal brain tissue and nonglial tumors.[21] CLE findings were comparable with standard H&E-stained slide assessments, supporting the utility of CLE in differential intraoperative diagnoses.

In a single-center feasibility study with an early version of the current ZEISS CONVIVO CLE system in 31 patients with a variety of brain tumors (mainly meningiomas and metastatic gliomas), the neurosurgeon-neuropathology team were able to identify high-grade gliomas and vascular neo-proliferation and to define tumor margins intraoperatively.[8] CLE provided sufficient resolution to achieve preliminary diagnosis without removal of biopsy samples.

In a case series based on 50 tumor resections, findings from intraoperative CLE correlated well with corresponding traditional histology in identifying pathognomonic cytoarchitectural features in various brain tumor types; in a blinded sub-analysis, 26 (92.9%) of 28 lesions were diagnosed correctly.[9]

A prospective surgical evaluation of real-time CLE in 74 patients provided histological specificities and sensitivities for gliomas and meningiomas that were comparable to those derived from standard assessments by frozen section histopathology.[10]

Supported by findings from preclinical, proof-of-concept data from animal studies,[5,22] these clinical data suggest that CLE with FNa contrast could allow real-time, interactive, intraoperative identification of brain tumor areas, which may provide improvements in the efficiency of decision making during neuro-oncological tumor resection.[4,10]

Beyond static 2D imaging

In addition to static two-dimensional image analysis, the Z-stack technology of current CLE systems allows intraoperative three-dimensional construction of time series and video loops to enable assessment of blood cell movements inside the brain vasculature and within brain tissue if oozing blood is present.[1,2,9] In addition, movement of tumor cells relative to one another and to normal brain cells during intraoperative squeezing can provide a further dimension for neuropathological assessment, by which movement of individual tumor cells in the border regions relative to cells at the tumor core can be assessed.[1]

Outlook

Overall, evidence from in vivo and ex vivo clinical studies conducted to date indicates that CLE shows promise for precise identification of tumor margins during assessment and resection procedures.[1,3] The possibility of real-time in vivo intraoperative neuropathological consultation could bring tangible benefits for the brain tumor surgery workflow,[1,4,11] and imaging technologies such as CLE could potentially lead to new diagnostic criteria for tumor characterization above and beyond those described using conventional two-dimensional histology.[5,12] In addition, optical imaging provides information that could potentially be leveraged to reveal previously unrealized features of disease and/or biochemical composition in neurosurgery.[23]

Please find further details regarding study design and findings in the **original articles** listed in the **reference list** at the end of this document.

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The publications below are based on the authors' own professional opinions or their study results. They do not necessarily reflect the opinions of ZEISS and may not be in line with the clinical evaluation or intended purpose of our medical devices. Therefore, suitability of clinical application for each recommendation should be carefully assessed by the concerned physician.

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