Intraoperative Fluorescence-Guided Resection of High-Grade Gliomas: A Comparison of the Present Techniques and Evolution of Future Strategies

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Key words
- 5-ALA
- Fluorescein
- Fluorescence-guided resection
- Malignant gliomas
- Neurosurgical procedures

Abbreviations and Acronyms
5-ALA: 5-Aminolevulinic acid
EOR: Extent of resection
FLIM: Fluorescence lifetime imaging microscopy
GBM: Glioblastoma multiforme
GTR: Gross total resection
ICG: Indocyanin green
MRI: Magnetic resonance imaging
PpIX: Protoporphyrin IX
QDs: Quantum dots
TR-LIFS: Time-resolved fluorescence spectroscopy

OBJECTIVE: Fluorescence guidance has demonstrated potential in maximizing the extent of high-grade glioma resection. Different fluorophores (fluorescent biomarkers), including 5-aminolevulinic acid (5-ALA) and fluorescein, have been examined with the use of several imaging techniques. Our goal was to review the state of this technology and discuss strategies for more widespread adoption.

METHODS: We performed a Medline search using the key words “fluorescence,” “intraoperative fluorescence-guided resection,” “intraoperative image-guided resection,” and “brain glioma” for articles from 1960 until the present. This initial search revealed 267 articles. Each abstract and article was reviewed and the reference lists from select articles were further evaluated for relevance. A total of 64 articles included information about the role of fluorescence in resection of high-grade gliomas and therefore were selectively included for our analysis.

RESULTS: 5-ALA and fluorescein sodium have shown promise as fluorescent markers in detecting residual tumor intraoperatively. These techniques have demonstrated a significant increase in the extent of tumor resection. Regulatory barriers have limited the use of 5-ALA and technological challenges have restricted the use of fluorescein and its derivatives in the United States. Limitations to this technology currently exist, such as the fact that fluorescence at tumor margins is not always reliable for identification of tumor-brain interface.

CONCLUSIONS: These techniques are safe and effective for increasing gross total resection. The development of more tumor-specific fluorophores is needed to resolve problems with subjective interpretation of fluorescent signal at tumor margins. Techniques such as quantum dots and polymer or iron oxide-based nanoparticles have shown promise as potential future tools.

INTRODUCTION
Gliomas account for nearly 80% of primary malignant brain tumors, contributing to approximately 13,000 deaths and 18,000 new cases annually in the United States (58). This devastating disease accounts for more years of life lost than any other type of tumor (58). Glioblastoma multiforme (GBM) is the most common type of glioma and carries a very poor prognosis. The current standard of care results in an average life expectancy of 14 months after diagnosis (58). The extent of resection in patients with GBM primarily determines the length of life expectancy. Improving intraoperative visualization and detection of residual tumor is vital to improving patients’ prognosis (2, 52). Unfortunately, the heterogeneity, similarity of tumor appearance under the operating microscope to the surrounding brain parenchyma, and diffusely infiltrative behavior of high-grade gliomas make complete tumor resection challenging and difficult to achieve (2, 15, 51). Traditional imaging techniques do not permit a complete intraoperative identification of residual tumor cells. The tumor-brain interface may be difficult to identify for selective tumor removal sparing surrounding brain parenchyma (2, 15, 52).

Resection of these gliomas can be facilitated by the application of fluorescence and computer-assisted neuroimaging modalities to provide more precise localization and removal of the tumor (2, 15, 47, 51, 52, 82). Several techniques that use fluorescent biomarkers have been investigated as a means to improve intraoperative navigation and identification of residual tumor. These developments permit improved identification of tumor tissue, facilitating gross total resection (GTR) assessed via the use of contrast-enhanced T1-weighted imaging (65); there has been no significant improvement, however, offered in patients’ long-term prognosis (2, 15, 51, 52, 58). The aim of this study was to provide a review and analysis of the advantages and limitations of intraoperative fluorescence-guided resection of high-grade gliomas and provide an insight for similar future studies.
METHODS
A systematic evidence-based review of the relevant published literature was completed. A Medline search was performed using the key words “fluorescence,” “intraoperative fluorescence-guided resection,” “intraoperative image-guided resection,” and “brain glioma.” These articles were filtered for those published in English, on human subjects, and dates from 1960 until present. This initial search revealed 267 articles. Each abstract and article was reviewed, and the reference lists from select articles were further evaluated for relevance pertaining to use of various modalities for fluorescence-guided surgery of high-grade gliomas, their intraoperative characteristics, impact on extent of resection, specificity, sensitivity, and clinical outcomes. A total of 64 articles included important information about the role of fluorescence in resection of high-grade gliomas and therefore were selectively included for our analysis.

RESULTS
Basic Mechanisms of Fluorescence
Electromagnetic radiation can excite electrons in atoms or molecules from their ground state (S₀) to a greater energy state (S₁). Excitation energy between 1.5 eV and 3.5 eV is required for aromatic organic molecules; this corresponds to wavelengths between 800 nm and 300 nm. Fluorescence occurs when electrons relax from their S₁ to S₀ state by emitting photons of light. Excitation light is of lower wavelength (i.e., greater frequency and higher energy) and the corresponding fluorescent radiation is of longer wavelengths (i.e., lower frequency and lower energy)—a phenomenon known as the Stokes shift (36, 82). This transfer typically happens in picoseconds to nanoseconds (71).

Fluorescence Lifetime
Fluorescence lifetime is the average amount of time a fluorophore remains in the excited state after excitation. Different molecular constituents of cells display intrinsic auto-fluorescence. As a result of the variability of cellular (e.g., cytosol, mitochondria) or tissue (e.g., normal, necrotic, neoplastic) environments in which these molecules are found, variations in fluorescence lifetimes can be used as a possible mechanism of contrast to distinguish tissue (6, 7, 30, 37, 56, 82, 87). Several groups have reported on the utility of fluorescence lifetime for delineating normal from pathologic tissue (11, 13, 61). A major advantage of using time-resolved fluorescence assessments using either spectroscopic or imaging devices is that lifetime assessments are independent of blood, irregular brain surfaces, or tissue illumination, unlike measurements of fluorescence intensity alone (3, 4, 11).

Time-resolved fluorescence spectroscopy (TR-LIFS) measures fluorescence lifetime, but rather than in imaging mode, it does so via a point spectroscopy device (6-8, 37, 47, 48, 82, 87). Butte et al. (6, 7) previously demonstrated the implementation of TR-LIFS to detect fluorescence lifetime differences between normal brain tissue and glioma ex vivo and more recently reported the potential of applying TR-LIFS for intraoperative diagnosis of gliomas. Normal cortex and white matter showed two fluorescence peaks at 390 nm and 460 nm, whereas the 390-nm emission peak was absent in low-grade gliomas (N = 5) and reduced in high-grade gliomas (N = 9) (6). These differences found in vivo suggest that TR-LIFS may function as potential adjuncts for facilitating delineation of the brain-tumor interface; however, additional studies are needed to validate its accuracy as an intraoperative tool for delineation of brain tumors.

Fluorescence lifetime imaging microscopy (FLIM) is an imaging technique used by Sun et al. (70) to determine the practicality of using an endoscopic fluorescence lifetime imaging for intraoperative identification of GBM. These authors evaluated the technique by validation between FLIM and magnetic resonance imaging (MRI), intraoperative gross judgment, and gross pathologic assessment. In this study, the FLIM instrument was adapted onto an endoscopic probe that allows the neurosurgeon to visualize tumor surface. The fluorescence intensity and lifetime centered at 460 nm (major emission band for nicotinamide adenine dinucleotide) were significantly weaker and longer, respectively, in GBM compared with normal parenchyma after ultraviolet excitation. This pilot study presented initial results on the potential application of FLIM for intraoperative tumor identification.

5-Aminolevulenic acid (5-ALA). Formation of ALA is the initial rate-limiting step in the porphyrin synthesis pathway that ultimately leads to heme synthesis. Exogenous administration of 5-ALA functions as a prodrug, which is then metabolized and promotes accumulation of fluorescent protoporphyrin IX (PpIX) in tissues through the heme biosynthesis pathway (83). Although malignant gliomas show an increased accumulation of PpIX, the exact mechanism responsible for excess PpIX production in neoplastic cells after the exogenous administration of 5-ALA is not yet fully understood. The main mechanism of accumulation of PpIX in malignant tissue initially was believed to be the result of 5-ALA crossing a disrupted blood-brain barrier. However, several studies have demonstrated that this is not the only mechanism, and that specificity for the malignant cells might exist. Stummer et al. (67) studied 144 biopsies obtained from 66 patients. The intensity of PpIX fluorescence was assessed spectrographically. Histopathologic analysis was conducted in terms of cellular density, MIB-1 labeling index (as an indicator of proliferative activity), and the area of CD-31 staining was measured to determine neovascularity. A multiregression model demonstrated that CD-31 staining was barely significant, meaning that neovascularity and BBB abnormality were not the only factors related to fluorescence accumulation.

Evidence suggests that PpIX accumulates more in greater-grade gliomas compared with low-grade gliomas because of increased proliferation, disruption of the blood-brain barrier, neovascularization, differential expression of membrane transporters, and intracellular enzymes (32, 46, 65, 67). Selective accumulation of ALA-induced PpIX between normal brain and tumor tissue provides necessary contrast and gradient for tissue delineation (25, 67, 83). Intraoperative imaging of PpIX fluorescence allows the neurosurgeon to visualize tumor and thus optimize the potential for maximal resection (Figures 1 and 2) (50, 67, 75, 79, 80).

Currently 5-ALA is not approved by the Food and Drug Administration for surgical resection of brain tumors; however, multiple studies have aimed to assess the effects of ALA fluorescence-guided glioma resection on the extent of gross total resection and progression-free survival.