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Review article

Activatable fluorescent probes in fluorescence-guided surgery: Practical considerations

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ABSTRACT

Fluorescence-guided imaging during surgery is a promising technique that is increasingly used to aid surgeons in identifying sites of tumor and surgical margins. Of the two types of fluorescent probes, always-on and activatable, activatable probes are preferred because they produce higher target-to-background ratios, thus improving sensitivity compared with always-on probes that must contend with considerable background signal. There are two types of activatable probes: 1) enzyme-reactive probes that are normally quenched but can be activated after cleavage by cancer-specific enzymes (activity-based probes) and 2) molecular-binding probes which use cancer targeting moieties such as monoclonal antibodies to target receptors found in abundance on cancers and are activated after internalization and lysosomal processing (binding-based probes). For fluorescence-guided intraoperative surgery, enzyme-reactive probes are superior because they can react quickly, require smaller dosages especially for topical applications, have limited side effects, and have favorable pharmacokinetics. Enzyme-reactive probes are easier to use, fit better into existing work flows in the operating room and have minimal toxicity. Although difficult to prove, it is assumed that the guidance provided to surgeons by these probes results in more effective surgeries with better outcomes for patients. In this review, we compare these two types of activatable fluorescent probes for their ease of use and efficacy.

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Contents

Abbreviations: ALA, aminolevulinic acid; CT, computerized tomography; EPR, enhanced permeability and retention; FDA, Food and Drug Administration; FRET, Föster (fluorescence) resonance energy transfer; gGlu, γ-glutamyl; GGT, γ-glutamyltransferase; HMRG, hydroxymethyl rhodamine green; ICG, indocyanine green; ISFA, intraoperative frozen section analysis; MRI, magnetic resonance imaging; PeT, photon induced electron transfer; RES, reticuloendothelial system; TBR, target-to-background ratio.

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1. Introduction

In vivo medical imaging technologies have seen numerous developments due to advances both in imaging devices and imaging probes.[1] Optical imaging is a type of *in vivo* imaging that uses fluorescent probes that emit light at a range of wavelengths when excited by light of a lower wavelength. Optical imaging has attracted attention as a non-invasive tool with numerous preclinical and clinical applications for oncologic analysis, including tumor detection, biomarker visualization, and vascular/lymphatic mapping.[2] A particularly promising application of optical imaging is fluorescence imaging during surgery, which has become one of the most rapidly adopted optical imaging methods.[3] Fluorescence imaging has the advantages of using low-cost, easy-to-use, portable equipment, with probes that have a high safety margin and a high sensitivity for cancer in the picomolar range.[3–7]

In cancer surgery, a major goal is to remove tumor as possible while preserving healthy tissues.[8] Negative tumor margins or the complete resection of tumors is important for improving survival.[9,10] Surgery to resect tumors is largely based on the surgeon's experience and ability to see anatomical features under the white light conditions of the operating theater.[8] Due to the low contrast between cancerous and normal tissues, accurately identifying the border between cancer and normal tissues may be difficult with the unaided human eye.[9,11,12] In addition, tiny foci (<2–3 mm) of cancer may be impossible to spot without the assistance of fluorescence imaging.[11–13]

Currently, the gold standard for determining tumor margins is intraoperative frozen section analysis (IFSA). IFSA has several limitations including the requirement for skilled personnel over a prolonged time, resulting in increased costs even while the method often is not accurate for positive margins.[9,15,16] It is estimated that IFSA adds approximately 30–53 min to surgical procedures, thus increasing anesthesia-related risks.[8,14] In addition to long processing times and insufficient sensitivity, only limited sampling of tissues is possible, which increases the possibility of false negative results leading to early recurrence.[15–17]

A number of imaging methods have been proposed to aid surgery. For instance, intraoperative CT and MRI have played a significant role in the field of neurosurgical surgery.[18,19] However, intraoperative whole body systems are costly, complex, require space, and their use interrupts the normal workflow of the surgical procedure, lengthening operative/anesthesia times.

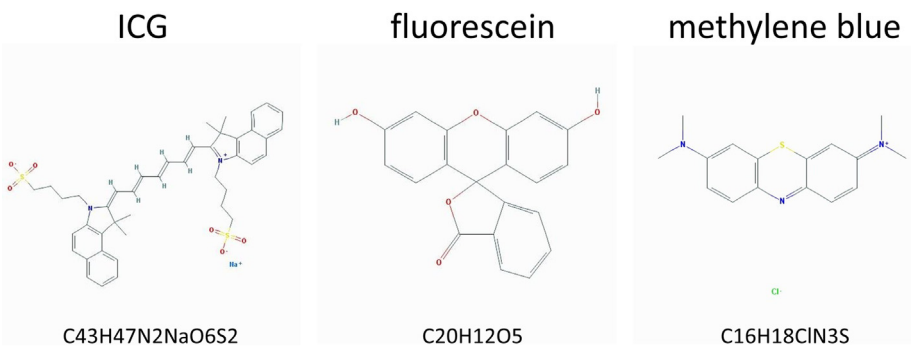
A more promising alternative is intraoperative optical fluorescence imaging, which is a real-time imaging technology that is increasingly used to aid surgeons in identifying surgical margins for tumor resection.[6,12] Because white light cameras are used in many operating rooms and endoscopy suites already, optical fluorescence imaging is easily integrated into the workflow of intraoperative surgery and endoscopy.[6,20,21]

Indocyanine green (ICG), methylene blue, and fluorescein are fluorescent probes approved by the United State (Scheme 1).

ICG is one of the most frequently employed near-infrared fluorescent probe. Throughout its history, ICG has maintained a high safety index.[22] ICG is routinely used to evaluate hepatic function and clears from tumors such as gliomas at a slightly lower rate than normal tissue.[22–24] When injected into the body, ICG increases fluorescence signal after albumin binding. However, it is difficult to design a targeted fluorescent probe with ICG because ICG loses fluorescence after covalent conjugation with proteins.[22]

In addition to ICG, a number of other dyes have been used for intraoperative guidance. Methylene blue is a near-infrared fluorescent agent but it has low quantum yield which hampers its clinical application.[25] Fluorescein has been shown to significantly improve resection of gliomas, yet like the others, it is not tumor-specific and can give false-negative or false-positive signals.[26,27] Moreover, its light has minimal tissue penetration *in vivo*. In Europe, 5-aminolevulinic acid (5-ALA) is an approved probe used to assist in tumor resection and has been shown to improve 6-month progression free survival in patients with malignant gliomas.[28,29] However, low specificity of fluorescent probes at tumor margins introduces error as the agents cannot differentiate tumors from reactive vascularity.[28–30] As the strategy of removing cancerous tissues during surgery faces limitations, it is becoming increasing important to further develop the next generation of fluorescent imaging probes.[2,9,31]

There are two types of fluorescent probes used for fluorescent imaging: "always-on" and "smart" or "activatable" probes.[12] Always-on probes continuously emit signal regardless of their relative proximity to or binding with target cells and they, therefore, accumulate both at the target and in background tissue.[32,33] Therefore, using always-on probes produces relatively lows target-to-background ratios (TBR), making it more difficult to visualize the tissue of interest. An adequate TBR is only reached after waiting a considerable time for probes in the background to clear, but at the same time, probes bound to the tumor will also begin to clear and produce a lower signal.[21,34] On the other hand, activatable probes remain undetected until they are turned on by specific enzymes or environmental conditions and emit signal, leading to increased contrast and sensitivity: a bright tumor against a dark background (Fig. 1).[35,36]



Scheme 1. Chemical structures and molecular formula of clinically used fluorophores.