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Advances in imaging probes and optical microendoscopic imaging techniques for early *in vivo* cancer assessment ☆

Numfon Khemthongcharoen [a,1], Romuald Jolivot [a,1], Santi Rattanavarin [a], Wibool Piyawattanametha [a,b,*]

[a] National Electronics and Computer Technology Center (NECTEC), Pathum Thani 12120, Thailand
[b] Advanced Imaging Research (AIR) Center, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

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ABSTRACT

A new chapter in the history of medical diagnosis happened when the first X-ray technology was invented in the late 1800s. Since then, many non-invasive and minimally invasive imaging techniques have been invented for clinical diagnosis to research in cellular biology, drug discovery, and disease monitoring. These imaging modalities have leveraged the benefits of significant advances in computer, electronics, and information technology and, more recently, targeted molecular imaging. The development of targeted contrast agents such as fluorescent and nanoparticle probes coupled with optical imaging techniques has made it possible to selectively view specific biological events and processes in both *in vivo* and *ex vivo* systems with great sensitivity and selectivity. Thus, the combination of targeted molecular imaging probes and optical imaging techniques have become a mainstay in modern medicinal and biological research. Many promising results have demonstrated great potentials to translate to clinical applications. In this review, we describe a discussion of employing imaging probes and optical microendoscopic imaging techniques for cancer diagnosis.

Contents

Abbreviations: US, ultrasound; CT, computed tomography; MRI, magnetic resonance imaging; SPECT, single photon emission computed tomography; PET, positron emission tomography; NIR, near infrared; IR, infrared; AFI, autofluorescence imaging; NADH, nicotinamide adenine dinucleotide hydrogenase; PDD, photodynamic diagnosis; PDT, photodynamic therapy; LEDs, light-emitting diodes; FLI, fluorescence lifetime imaging; BLI, bioluminescence imaging; CCD, charge-coupled device; NPs, Nanoparticles; UV, ultraviolet; QDs, Quantum dots; LRET, luminescence resonance energy transfer; FOV, field of view; PSF, point spread function; DAC, dual-axis confocal; MEMS, microelectromechanical systems.

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* Corresponding author. Tel.: +66 87 936 5000.
E-mail address: wibool@gmail.com (W. Piyawattanametha).

[1] Authors contributed equally.

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1. Introduction

Currently, standard screening of cancer involves the use of white light endoscopes to screen tissue abnormalities meaning that practitioners can rely only on their visual perceptions [1]. As a result of this limited information, identification of these tissues is challenging because precancerous lesions do not present many changes in colors or morphological differences compared to healthy tissue under white light illumination. Then, the excised lesions need to be processed in laboratory. The interpretive accuracy of the excised lesions is affected by artifacts such as paraffin embedding and histochemical staining resulting from the tissue sectioning procedures. These conditions are time consuming and delay the potential treatment to patients. Therefore, many research projects are underway to develop new methods to perform *in vivo* imaging and link acquired images with pathology in order to deliver *in vivo* diagnosis in real-time and without risks related to biopsy procedure. Combination of new diagnostic imaging devices [2] and targeted biomarkers enable delivery of high resolution, accuracy, reliability, sensitivity, and specificity information about tissues to potentially avoid unnecessary biopsies (resulting in a gain in terms of diagnosis time, accuracy and cost saving, and patient satisfaction along with a higher chance of patient compliance). The ultimate goal is to translate these combination techniques to clinics [3]. This review presents the current progresses and challenges in both imaging probes and optical microendoscopes for early cancer assessment. With this early assessment achieving from these integrated approaches, it allows screening for patients who would potentially respond to therapy and have higher possibilities of a favorable treatment outcome.

2. Nanoprobes for optical imaging

Advancement in light microendoscopy currently furnishes real-time and non-invasive imaging in living subjects with sub-cellular to cellular resolutions. In comparison to other medical macro-scale (millimeter to centimeter range) imaging modalities such as ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), and radionuclide-based imaging (single photon emission computed tomography, SPECT and positron emission tomography, PET), optical imaging tools are non-ionizing, inexpensive, image signal quantifiable, micro-scale (submicron to micron range) imaging, and safe for repeated use [4,5]. Among available optical imaging instruments used in clinics, confocal microendoscopes have the most potential to perform standard histopathological images such as those found in paraffin-embedded tissue biopsy examination with sub-cellular to cellular resolution, which covers the superficial layer of the epithelial tissue *in vivo*. Furthermore, this technique permits an inexpensive real-time histopathological examination *in vivo* during the endoscopic procedure. Reducing the number of tissue biopsies and thus diminishes the risk of surgical site infection [6]. Confocal microendoscope can be operated in reflectance imaging mode by using non-absorbed illuminated light in near infrared (NIR) wavelengths, ranging from 700 to 900 nm. Then, scattered light from the targeted cells and tissues is collected and analyzed. Fluorescence imaging mode uses a confocal microendoscope in combination with fluorescent contrast agents. This imaging mode is widely used in cancer imaging to enhance imaging resolution and to eliminate the interference reflected light at the air–liquid interface [7,8]. Some common dyes used in endoscopic investigation that have fluorescence emission properties such as crystal violet [6,9], methylene blue [6,7], proflavine [6,10–12], acridine orange [13,14], and fluorescein [13–16] have been extensively applied on the tissue surface to reveal abnormal cells and tissue in confocal microendoscopic investigations. Because the molecular sizes of these contrast agents are small, they easily penetrate into the tissue masses and localize intra- and extra-cellularly. The dyes can be applied *via* topical application or intravenous injection. The different staining ability of various cells and tissue components including nucleic acid, lipid, cytosol, and proteins creates contrast image of cells and tissues. Abnormal cell or tissue morphologies can be characterized guiding the cancer lesion area for surgical treatment promptly during the endoscopic examination. Therefore, confocal microendoscopy is currently a prominent technology used in modern cancer investigation allowing cancer diagnosis and surgical treatment at the same time. Diagnostic applications directly linked to specific therapeutic approach are termed as "theranostics" [17]. The sections below will cover various nanoprobes employing in optical imaging modalites starting from the basic non-specific contrast agents to the latest nanoprobes currently being developed in modern optical theranostic imaging applications.

2.1. Contrast agents for optical imaging

Optical imaging instruments generally utilize the imaging probes, which have fluorescence or bioluminescence properties to enhance image contrast. The simplest form of contrast agents can be found innately as autofluorescences in cells and tissues. However, sensitivity and specificity of those innate fluorescences are not sufficient for detection of tumor lesions in early stage. Therefore, exogenous fluorophores and bioluminescences have been combined with various bio-chemical and bio-metal materials to invent novel, highly sensitive, and specific imaging probes for modern cancer administration.

2.1.1. Fluorescence-based optical contrast agents

Florescence is the most common quantifiable optical contrast agents used in *in vivo* cancer imaging. Fluorescent lights are emitted from fluorophores when they absorb energy from an external excitation light source. Photon energy activates electrons of a fluorophore from its ground stage to an excited stage before returning to their relaxation period after fluorescent light emission. Each fluorophore has its own specific absorbed excitation and emission optical wavelengths. Therefore, fluorescence signals can be specifically utilized by determining and collecting optical wavelengths according to the specific excitation and emission profile of each fluorophore [18]. Fluorophores can be found naturally in living cells (endogenous fluorophores) or in the chemical agents applied from outside the cell (exogenous fluorophores). Both endogenous and exogenous fluorophores have been ubiquitously used as contrast agents for *in vivo* cancer detection based on fluorescence lifetime imaging (FLI) techniques.

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