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Near-infrared fluorescence (NIRF) imaging in breast-conserving surgery: Assessing intraoperative techniques in tissue-simulating breast phantoms

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Abstract

Purpose: Breast-conserving surgery (BCS) results in tumour-positive surgical margins in up to 40% of the patients. Therefore, new imaging techniques are needed that support the surgeon with real-time feedback on tumour location and margin status. In this study, the potential of near-infrared fluorescence (NIRF) imaging in BCS for pre- and intraoperative tumour localization, margin status assessment and detection of residual disease was assessed in tissue-simulating breast phantoms.
Methods: Breast-shaped phantoms were produced with optical properties that closely match those of normal breast tissue. Fluorescent tumour-like inclusions containing indocyanine green (ICG) were positioned at predefined locations in the phantoms to allow for simulation of (i) preoperative tumour localization, (ii) real-time NIRF-guided tumour resection, and (iii) intraoperative margin assessment. Optical imaging was performed using a custom-made clinical prototype NIRF intraoperative camera.
Results: Tumour-like inclusions in breast phantoms could be detected up to a depth of 21 mm using a NIRF intraoperative camera system. Real-time NIRF-guided resection of tumour-like inclusions proved feasible. Moreover, intraoperative NIRF imaging reliably detected residual disease in case of inadequate resection.
Conclusion: We evaluated the potential of NIRF imaging applications for BCS. The clinical setting was simulated by exploiting tissue-like breast phantoms with fluorescent tumour-like agarose inclusions. From this evaluation, we conclude that intraoperative NIRF imaging is feasible and may improve BCS by providing the surgeon with imaging information on tumour location, margin status, and presence of residual disease in real-time. Clinical studies are needed to further validate these results.

Synopsis: *Near-infrared fluorescence (NIRF)-based tumour imaging has great potential for improving breast-conserving surgery. In this report, intraoperative NIRF applications were preclinically evaluated using tissue-simulating breast phantoms equipped with fluorescent tumour-like inclusions of different size and shape in breast-conserving surgery simulations.*

Keywords: Human breast cancer; Near-infrared fluorescence; Tissue-simulating phantoms; Intraoperative imaging

Introduction

Breast cancer is the most frequent malignancy in women worldwide with an estimated 1.4 million new cases in 2010.[1] Breast-conserving therapy (BCT), consisting of breast-conserving surgery (BCS) followed by radiation therapy, has become the standard treatment for T1–T2

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breast tumours and is generally regarded as sufficient for this subset of patients.[2] Unfortunately, a majority of studies on the surgical margin status after BCS have shown that positive margins are detected in 20–40% of patients, necessitating additional surgical intervention or radiotherapy.[3] Two major points for improving outcome after BCS involve (i) a more reliable intraoperative tumour localization and (ii) improved real-time feedback on the presence of possible residual disease during or after excision of the tumour.[4] Intraoperative application of an optical imaging technique known as near-infrared fluorescence (NIRF) imaging may improve the clinical outcome of BCS.[3,5]

Near-infrared fluorescence imaging

In recent years, significant progress has been made in the development of optical imaging systems and fluorophores for clinical applications.[6,7] Several animal[5,8–10] and clinical[11–15] studies have shown the potential clinical use of NIRF imaging to improve the therapeutic outcome of surgery. Compared to light in the visible spectral range (400–650 nm), application of near-infrared (NIR) light minimizes absorption by physiologically abundant molecules such as hemoglobin and lipids, which increases penetration depth.[16,17] Additionally, autofluorescence (the intrinsic fluorescence signal present in all living cells due to various normal metabolites and tissue constituents) is strongly reduced in the NIR spectral range. Taken together, these aspects of NIR light make it particularly suitable for use in intraoperative optical imaging applications. However, clinical application of NIRF imaging in BCS is currently limited to the non-specific intraoperative detection of the sentinel lymph node.[11,12,14,18–20]

Tumour-targeted near-infrared fluorophores

With the introduction of clinical grade tumour-targeted NIR fluorophores, NIRF imaging may be extended towards the intraoperative detection of the primary tumour.[10] Several target molecules have been identified for breast cancer that may be of value for such an approach, including Her2/neu receptor,[9,21,22] vascular endothelial growth factor (VEGF) receptor,[23,24] endothelial growth factor (EGF) receptor[25] and folate receptor-α.[26]

In tumour-targeted NIRF imaging, a tumour-targeted NIR fluorophore is administered several hours or days prior to the imaging procedure. Subsequently, an external laser is used to irradiate the breast with light in the NIR spectral range (650–900 nm).[17] Upon excitation, the fluorophore will release photons of a higher NIR wavelength. Because NIR light is invisible to the naked eye, a dedicated optical imaging system is necessary to capture the NIR signal from the surgical field and digitally convert it to a visible image. Recently, we and our co-workers developed a multispectral NIRF intraoperative camera system that is suitable for intraoperative use with NIR fluorophores.[27]

Simulation of NIRF-guided breast-conserving surgery

In the current preclinical study, we evaluated intraoperative NIRF imaging applications in a simulated clinical setting as a step-up toward NIRF-guided BCS. To this end, we used tissue-simulating gelatin-based breast phantoms that mimic the optical properties of normal breast tissue.[28,29] Tumour-like fluorescent inclusions of different size and shape were positioned at predefined sites in the phantoms, allowing for simulation of (i) preoperative tumour localization, (ii) real-time NIRF-guided tumour resection and (iii) intraoperative macroscopic margin assessment. The tumour-like inclusions contain the non-specific NIR fluorophore indocyanine green (ICG), to simulate for the use of tumour-targeted near-infrared fluorophores in BCS. Currently, ICG (absorption and emission maximum at ~780 and ~820 nm, respectively) is one of the few FDA-approved NIR fluorophores available for clinical use.[9] Sevick-Muraca et al. have previously shown the feasibility of NIRF imaging following microdose administration of ICG.[12] Although ICG in itself is non-specific, their findings suggest that comparable microdose concentrations can be used to label cancer cells with tumour-targeted NIR fluorophores for intraoperative NIRF imaging. Importantly, new fluorophores in the NIR spectral range are currently being developed, e.g. IRDye® 800CW, with properties more promising for intraoperative use compared to ICG.[25]

Material and methods

Assessment of ICG fluorescence self-quenching in agarose

Because increasing concentrations of ICG may not correspond to an increased fluorescence signal due to self-quenching of ICG, different concentrations of ICG in agarose were evaluated for fluorescence activity.[29,30] Briefly, an ICG stock solution was serially diluted in 10 ml sterile water (ranging from 0.5 uM to 350 uM ICG), after which 2% agarose was added. The mixture was then heated to 70 °C and stirred until the agarose was completely dissolved. After solidification of the agarose mixture for 15 min at 4 °C, NIRF epi-illumination imaging was performed to determine maximum photon counts/sec (settings: exposure time: 1000 ms, excitation: 780 nm, emission: 820 nm).

Assessment maximal penetration depth of ICG fluorescence

In order to determine the maximal penetration depth of the NIRF signal, a cubic fluorescent inclusion of 5 × 5 × 5 mm containing 14 μM ICG was positioned in phantom tissue at a depth of 30 mm. Subsequently, the surgeon excised 3–4 mm layers of phantom tissue towards the inclusion (remaining depths were 27, 24, 21, 18, 15, 11, 7,