Preoperative Mapping of Nonmelanoma Skin Cancer Using Spatial Frequency Domain and Ultrasound Imaging

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Rationale and Objectives: The treatment of nonmelanoma skin cancer (NMSC) is usually by surgical excision or Mohs micrographic surgery and alternatively may include photodynamic therapy (PDT). To guide surgery and to optimize PDT, information about the tumor structure, optical parameters, and vasculature is desired.

Materials and Methods: Spatial frequency domain imaging (SFDI) can map optical absorption, scattering, and fluorescence parameters that can enhance tumor contrast and quantify light and photosensitizer dose. High frequency ultrasound (HFUS) imaging can provide high-resolution tumor structure and depth, which is useful for both surgery and PDT planning.

Results: Here, we present preliminary results from our recently developed clinical instrument for patients with NMSC. We quantified optical absorption and scattering, blood oxygen saturation (StO_2), and total hemoglobin concentration (THC) with SFDI and lesion thickness with ultrasound. These results were compared to histological thickness of excised tumor sections.

Conclusions: SFDI quantified optical parameters with high precision, and multiwavelength analysis enabled 2D mappings of tissue StO_2 and THC. HFUS quantified tumor thickness that correlated well with histology. The results demonstrate the feasibility of the instrument for noninvasive mapping of optical, physiological, and ultrasound contrasts in human skin tumors for surgery guidance and therapy planning.

Key Words: Skin cancer; optical imaging; ultrasound; optical and blood parameters; surgery; PDT.

Nonmelanoma skin cancers (NMSCs), which include basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), are the most common human cancer with more than one million cases every year, and the incidence rate has increased dramatically. Although they rarely metastasize, they can present significant morbidity especially for cases in cosmetically sensitive areas, such as the face. The standard of care for NMSCs is usually surgical excision or Mohs micrographic surgery. Tumors may show multifocal, widespread disease, and suspicious lesions at deeper locations may be present. Typically, biopsies are performed to guide surgeons but can be time-consuming and costly, and the analyzed sections may not be representative of the whole tumor. After surgical removal of the tumor, there may still be residual tumor at the margins, which can result in high-recurrence rates. Thus, the surgeon needs to decide on how much to excise and how deep to go during surgery. Surgery can benefit from prior knowledge of size and depth for more accurate lesion removal. An imaging tool that can provide guidance and thereby reduce recurrence rates, operation times, cost, and the need for multiple biopsies would be highly desired.

Depth and size information can also provide useful information for selecting the appropriate therapy. Topical 5-aminolaevulinic acid (ALA)–based photodynamic therapy (ALA-PDT) has become an attractive treatment option especially for cases with multiple sites and large areas (1,2). ALA-PDT uses topical application of the prodrug ALA that is converted into the photosensitizer (PS) protoporphyrin IX (PpIX), which is activated by light in the presence of oxygen for local tissue destruction. For superficial NMSCs, ALA-PDT has efficacy close to surgery with sometimes better cosmetic and functional outcomes. However, the efficacy is limited for thicker and deeper tumors (3–6). Thus, tumor size information can allow for a better PDT planning.

Acad Radiol 2014; 21:263–270

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http://dx.doi.org/10.1016/j.acra.2013.11.013

The PDT dose is related to the light, PS distributions and the available oxygen. Accurate dosimetry, which takes into account individual differences in light, PS dose distributions, and oxygenation levels, is needed for optimized PDT. In most clinical practice, light dose is the prescribed incident fluence rate and is usually fixed. Because light propagation in tissue is strongly affected by the tissue optical properties, local light dose can be significantly different than prescribed dose. Tumors can show significant intra- and interlesion heterogeneity with respect to optical absorption and scattering parameters, which may result in considerable intra- and interlesion variations in the deposited light dose (7). Similarly, intra- and intertumor PS distribution can show significant heterogeneity (8). Thus, knowledge about the spatial distributions of PS content is desired for PDT dose optimization (8). Because oxygen is critical for PDT and because PDT itself can induce significant oxygen depletion that can result in treatment failures, one needs to know about the available oxygen in the target tissue before PDT and how the oxygen is being consumed during PDT (9,10). Tissue oxygenation is substantially dependent on vascular parameters, such as blood oxygen saturation (StO_2) and blood volume (7,11). Thus, assessing these parameters can provide quantitative metrics for PDT dosimetry and response.

Spatial frequency domain imaging (SFDI) can quantify both optical absorption and scattering during reflectance imaging mode (12). Knowledge of the optical parameters can allow modeling the light dose distribution within the treatment field, whereas multiwavelength absorption enables the quantification of oxy-, deoxy-, and total hemoglobin concentrations related to blood volume and tissue StO_2 as shown in the recent proof-of-principle study of imaging skin lesions, which were located on easily accessible places on the body, such as the arms, legs and torso (13). In addition to PDT dosimetry, these parameters can provide intrinsic contrast enhancement and complement the existing imaging contrast before surgery for improved tumor demarcation.

Several noninvasive imaging modalities have been applied for quantifying the structure of skin tumors. Conventional ultrasound is a well-established imaging modality and is widely used today in preclinical and clinical settings (14,15). It is noninvasive and does not use radiation harmful to the human body. Its use to examine and assess the skin is relatively new (15,16). Compared to conventional ultrasound machines, high frequency ultrasound (HFUS) uses higher frequency ($\geq$20 MHz) sound waves to obtain high-resolution ($\sim$50 μm) images and relatively deep penetration depth for skin imaging (>2 mm) (15) without creating any additional safety issues. HFUS can provide information regarding skin structure (thickness of epidermis, dermis, etc.) and lesion thickness, which can guide optical imaging for improved accuracy, as demonstrated recently for guiding fluorescence imaging (17). Because of its high resolution, HFUS has recently shown promise for guiding Mohs surgery of NMSCs (15). Moreover, it was shown that prePDT tumor thickness strongly predicts the probability of local control of NMSCs (18). Thus, there exists a need for routine evaluation of tumor thickness at prePDT.

In this work, we present two interesting clinical cases from our ongoing clinical trial where we used SFDI and HFUS imaging for quantifying optical, vascular, and tissue structure parameters in patients with NMSCs located in imaging-wise challenging areas around the head and neck. Optical absorption (μ_a), scattering (μ_s'), and vascular parameters of StO_2 and THC quantified by SFDI showed clear tumor contrast when compared to the surrounding normal tissue, whereas HFUS imaging accurately quantified the tumor thicknesses. These noninvasive imaging results were qualitatively supported by the ex vivo analysis of hematoxylin and eosin (H&E) staining. Thus, we conclude that the noninvasive SFDI and the ultrasound imaging can provide quantitative contrasts and therapeutic metrics in NMSCs for surgical guidance and PDT planning at the clinical settings.

MATERIALS AND METHODS

Clinical Spatial Frequency Domain and Ultrasound Imaging Systems

We have initiated a clinical trial under the institutional review board–approved protocol #I226912, and informed consent was obtained from all patients before the measurements. The aim of this pilot study was to demonstrate noninvasive quantification of optical parameters, StO_2, blood volume, and thickness of NMSCs before surgery and to establish these techniques for future clinical trials involving PDT. In this study, patients with biopsy-proven nonmelanoma cancer lesions designated to be removed through Mohs micrographic surgery were enrolled.

A clinic-friendly SFDI system was constructed as shown in Figure 1. Figure 1a shows the complete unit at the clinical setting, whereas Figures 1b and 1c shows the picture and schematic diagram of the imaging head. The instrument consisted of four high-power, compact light-emitting diodes (LEDs), LCS series, each centered at 590 nm, 630 nm, 660 nm, and 740 nm, (Mightex, Toronto, Ontario, Canada). A four-channel LED controller (Mightex) sequentially selected the desired excitation wavelength, and light was directed through a liquid light guide to a projector (Light Commander; Logic PD, Inc., Minneapolis, MN, USA) with a digital micromirror device (DMD) module having 1024 $\times$ 768 pixel resolution. The DMD module generated the appropriate sine wave patterns with three different phases (0, $2\pi/3$, $4\pi/3$) and 11 spatial frequencies from 0 to 5 cm^{-1}. The patterns were projected onto the skin surface and reflected light was collected with the charge-coupled device (CCD) cameras. The cameras were focused on the same field of view the projector was illuminating (22 $\times$ 22 mm^2). A rigid light shield with an imaging window blocked room light and maintained a fixed distance to the target tissue. The system contained two CCD cameras separated by a 685 nm dichroic mirror (67-085; Edmund Optics, Barrington, NJ, USA) for imaging