Volumetric Multispectral Optoacoustic Tomography for 3-Dimensional Reconstruction of Skin Tumors: A Further Evaluation with Histopathologic Correlation

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TO THE EDITOR

Surgical extirpation is the most effective method to treat non-melanoma skin cancers (NMSCs), but it is usually cosmetically undesirable due to the large safety margins (Gualdi et al., 2015; Vuyk and Lohuis, 2001). Mohs micrographic surgery is ideal but time consuming (Tolkachjov et al., 2017). Thus, there is a clinical need for a preoperative imaging tool to guide surgery and map deep-penetrating NMSC 3-dimensionally. Skin cancer screening may begin with the aid of adjunctive tools for diagnosis, such as dermoscopy and reflectance confocal microscopy (Guitera et al., 2012; Malvehy and Pellacani, 2017), optical coherence tomography (Mogensen et al., 2009), and the sequential treatment. These imaging tools, however, lack penetration depth and specificity, making them unsuitable for unambiguous differentiation of certain skin structures, for example, melanin and blood vessels (Guitera et al., 2016).

We have demonstrated the clinical use of volumetric multispectral optoacoustic tomography (vMSOT) equipped with handheld detectors for 3-dimensional reconstruction of skin tumors in a pilot study (Supplementary Figure S1 online) (Attia et al., 2017; Chuah et al., 2017). This label-free, noninvasive imaging technique is based on real-time optoacoustic sensing of tissue absorbers, including hemoglobin, melanin, and lipids, which emit tiny ultrasound vibrations in response to absorption of photons from a laser beam (Ford et al., 2016). By capitalizing on the distinct spectral profiles of tissue biochromes, vMSOT is further able to spatially map their distribution in a volumetric (3-dimensional) space to render high-resolution morphology and vasculature information of cutaneous lesions. The acquired vMSOT images can be visualized in real-time during acquisition for anatomical navigation, while spectral unmixing for specific absorbers

Abbreviations used: BCC, basal cell carcinoma; NMSC, non-melanoma skin cancer; vMSOT, volumetric multispectral optoacoustic tomography

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Figure 1. Clinical and vMSOT images of non-melanoma skin cancer on representative patients. (a) BCC on the left cheek of patient 19, clinical image of lesion showing heterogeneous pigmented nodule. (b) H&E staining photomicrograph of lesion after excision showing nodules of basaloid cells. White scale bar = 1 mm. (c) Reflectance confocal microscopy image of the lesion during diagnosis showing nests of tumor cells in the dermis with bright white spots indicating melanin. White scale bar = 500 μm. (d) Maximum intensity projections of the vMSOT images along different projections (xy, yz, and xz) and the 3-dimensional rendering of the entire lesion. Yellow signals indicate melanin in the lesion while the red signals indicate the oxy-hemoglobin representing blood vessels in the lesion. The white lines drawn indicate the distance from which the collective vMSOT signals were plotted shown in for tumor (e) length and (f) depth. The tumor length and depth were then determined from the distance between the non-baseline values of the plot. a.u., arbitrary units. (g–l) BCC cases on patients 17 and 20. Maximum intensity projections of the vMSOT images of BCC cases in (g, h) Caucasian patient with Fitzpatrick skin type II (patient 17) and (i, j) Asian patient with Fitzpatrick skin type IV (patient 20).
lasts a number of seconds for each dataset off-line on a typical desktop. Herein, we aim to evaluate the reliability of vMSOT in the assessment of NMSC dimensions by correlating with histologic measurements of surgically resected NMSC and identify its limitations for in vivo clinical use. The vMSOT dimensions of the lesions were measured from their melanin or hemoglobin absorbers, depending on the lesion's pigmentation in a mixed ethnicity.

The maximum intensity projections show the different views of the skin lesion from the top (xy) top and their cross-sectional views (yz and xz). The clinical image of BCC on (h) patient 17 showed an erythemous plaque (blue arrow) on the left forehead, while the BCC on (k) patient 20 appeared as a pigmented plaque on the right forehead. The 3-dimensional MSOT rendering of both BCCs in (i) Fitzpatrick skin type II skin (patient 17) and (l) Fitzpatrick skin type IV skin (patient 20) are also shown. The BCCs were removed via Mohs micrographic surgery. Black scale bars = 10 mm; white scale bars on vMSOT images = 3 mm. BCC, basal cell carcinoma; H&E, hematoxylin and eosin; vMSOT, volumetric multispectral optoacoustic tomography.

Figure 2. Correlation and Bland–Altman plots for histology and vMSOT tumor depth and length measurements. Scatter plot of the tumor measurements (a) depth and (b) length by histology and vMSOT in the same tumor samples. The solid line indicates the regression line of the plot, while the dotted line indicates perfect agreement as reference. Less variability is seen between histologic and vMSOT measurements of tumor depth compared with their measurements of tumor length. Bland–Altman plot shows agreement of tumor (c) depth and (d) length measurements in histologic specimen (reference standard) and by vMSOT imaging. The differences between the two techniques are plotted against the means of the measurements. One of 25 tumors (4%) lies outside the limits of agreement for tumor depth; mean difference, −0.02 mm; 95% limits of agreement, −1.08 mm, 1.13 mm. One of 20 tumors (5%) lies outside the limits of agreement for tumor length; mean difference, −0.121 mm; 95% limits of agreement, −2.62 mm, 2.38 mm. The dashed lines indicate the limits of agreements and the solid line represent the mean difference in measurements between the two techniques. Scatter plot display of the differences in measurements between histology and vMSOT for the tumor (e) depth and (f) length to test the bias on the method of surgery represented. The lines represent the mean and standard deviation values of each scatter plot. vMSOT, volumetric multispectral optoacoustic tomography.
Population. Patient demographics (Supplementary Table S1 online) and image processing methodology are described in the Supplementary Materials online.

In one representative basal cell carcinoma (BCC) (patient 19; Figure 1a–1c, Supplementary Table S1), vMSOT images of the BCC were acquired, showing the lateral views and its 3-dimensional projection (Figure 1d). Distributions of the melanin and oxy-hemoglobin signals representing blood vessels could be visualized clearly. The tumor dimensions were then extracted from both melanin and hemoglobin signal distributions along the longest and deepest infiltration axes, represented by dotted white lines to give tumor length (7.42 mm; Figure 1e) and depth (3.04 mm; Figure 1f); correlating well with the excision length of 8.13 mm and depth of 2.50 mm.

Different subtypes of BCC on different skin phototypes were studied. Patient 17 with Fitzpatrick skin type II presented with an erythematous BCC plaque on his forehead (Figure 1g–1i). Most BCCs appear pigmented in people of color (Gloster and Neal, 2006, Kim et al., 2009), such as patient 20 (Fitzpatrick type IV) (Figure 1j–1l). Because vMSOT can differentiate the spectral signatures of oxy- and deoxy-hemoglobin from melanin, both pigmented and non-pigmented NMSCs can be imaged. The erythematous nature of the BCC on patient 17 was manifested as a strong congregation of oxy-hemoglobin signals on the superficial skin surface (Figure 1g) contrasting with the adjacent normal skin, whereby the dermal vasculature was ordered (Supplementary Figure S2 online).

A statistically significant correlation for both tumor depth and length was found between vMSOT and histologic analysis (r = 0.90, P < 0.0001 and r = 0.85, P < 0.0001, respectively, Figure 2a, 2b). The Bland–Altman plots showed that the lower and upper limits of agreement between the two measurement techniques of tumor depth were −1.47 mm and 0.76 mm, respectively, while they were −2.95 mm and 1.35 mm, respectively, for tumor length (Figure 2c, 2d). The Bland–Altman data also suggest that the tumor depth measurements agreed better than tumor length with a smaller standard deviation (0.57 vs. 1.10 mm for tumor depth and length, respectively). No significant difference was found between the differences in measurements between histology and vMSOT via excision and Mohs micrographic surgery. The differences in measurements between histology and vMSOT showed no significant bias towards the type of surgery (P = 0.17 and P = 0.38, tumor depth and length, respectively) (Figure 2e, 2f) and type of NMSC (P = 0.70 for tumor depth) (Supplementary Figure S3 online). Notably, there were only a few cases of squamous cell carcinoma represented in the statistical analysis, as only 20% of all NMSCs are squamous cell carcinoma (Eisemann et al., 2014).

Our results show that vMSOT is accurate in measuring tumor dimensions irrespective of the different types of NMSCs and skin phenotype. However, the accuracy can be compromised by the limited field of view of the vMSOT scanner and superficial location of the tumor (<0.5 mm depth). Case 22 reported a histologic depth of 0.47 mm, indicating that vMSOT was less accurate at very shallow depths, which can be attributed to the anisotropic resolution at the peripheral regions of the field of view of the matrix array detector (Ford et al., 2016). The lower detection limit of the vMSOT may lie between 0.47 and 1.28 mm, the latter being the smallest depth measured by vMSOT in this study (Supplementary Table S1). In case 16, the tumor depth measured by vMSOT exceeded the histologic measurement by a factor of ~5 (Supplementary Table S1). The disparity between the measurements may be attributed to the raised scaly plaque of the BCC, which may be present during the in vivo vMSOT imaging session, but could have dropped off during histologic processing.

Case 18, which lies below the lower limit of agreement of tumor length, yielded a vMSOT measurement twice the tumor length measured by histology. The ulceration and raised edges of the tumor coupled with its inaccessible location between the nose and lip could have contributed to the discrepancy (Supplementary Figure S4a online). Notably, the vMSOT depth was measured from the skin surface to the vascular structure, which corresponded to Mohs micrographic surgery depth. Visualizing the macrovasculature surrounding the NMSC may aid in the complete removal of the tumor.

The limitations of this study include the relatively small sample size, although it is generally sufficient to determine the agreement of tumor dimensions between the histologic and vMSOT measurements. Additionally, the 10 × 10 × 12 mm effective field of view of the MSOT configuration and the handheld probe geometry made it difficult to access certain curved areas on the face, thus limiting the number of NMSCs that could be studied.

The demonstration of vMSOT accuracy in determining tumor dimensions is pertinent, reinforcing the potential of its preoperative use in aiding dermatologic surgeons in margin demarcations to reduce the number of steps required in Mohs micrographic surgery. Furthermore, vMSOT offers a unique volumetric approach in visualizing the tumors noninvasively, thus offering significant diagnostic value.

This is a prospective, clinic-pathological and imaging correlation study conducted at the National Skin Centre, Singapore and it was sanctioned by the Domain Specific Review Board (DSRB) of National Health Group, Singapore (Ref No. 015/00462). Patients were imaged in compliance with respective institutional approvals and with written, informed consent.

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CONFLICT OF INTEREST
The authors state no conflict of interest.

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TO THE EDITOR

Intradermal injection of IL-23 and topical application of imiquimod (IMQ) are two widely adopted murine models of psoriasis. Both models result in psoriasiform dermatitis (PsD) in mice that resembles human psoriasis (van der Fits et al., 2009; Zheng et al., 2007). CCR6 is important for epidermal trafficking of IL-17/22-producing cells (Mabuchi et al., 2013) and is required for the development of PsD in the IL-23 injection model since CCR6-deficient knockout (CCR6KO) mice fail to show significant dermal inflammation (Hedrick et al., 2009). Using the IMQ model, however, Cochez et al. (2017) recently reported that CCR6 KO mice were still able to develop psoriatic lesions after application of a specific version of IMQ called Aldara (3M Pharmaceuticals, Maplewood, MN). Although epidermal IL-22 production was decreased, the authors found relatively little morphological change in the skin in CCR6KO versus wild-type (WT) control.

Other recent reports, however, suggest that CCR6 is critical for development of IMQ-mediated PsD. First, Robert et al. (2017) show that monoclonal antibodies to human CCR6 in a knock-in genetic model effectively block Aldara-mediated PsD. Second, using a small molecule antagonist of CCR6, Campbell et al. (2017) found that this inhibitor is effective in both IL-23- and IMQ-mediated PsD models in blocking skin inflammation. In the latter report, a generic version of IMQ (Fougera, Melville, NY) was used.

The possibility exists that the reason for the apparent differences in the importance of CCR6 lies in other ingredients in different commercial preparations of IMQ cream. For example, the Aldara vehicle alone can induce caspase-1-dependent pyroptosis of keratinocyte and concomitant pro-inflammatory cytokines secretion (Walter et al., 2013). To test this possibility and explore the role of CCR6 in the IMQ model, we applied a non-Aldara preparation of IMQ cream to CCR6KO mice and assessed the degree of PsD by histologic and immunologic criteria.

Mice were treated with 5% IMQ cream (Taro Pharmaceuticals, Hawthorne, NY) from day 0 to day 6 on each ear and then euthanized on day 7 (Figure 1a). Ear swelling, a marker for dermal inflammation and corresponding edema (Hedrick et al., 2009), was consistently reduced in CCR6KO versus WT mice throughout the entire course of the experiment (Figure 1a). Ear skin epidermal hyperplasia in the CCR6KO mice was reduced by 80% versus WT mice (Figure 1b–c). Quantitative reverse transcriptase PCR showed that mRNA expression of IL-17A, IL-17F, and IL-22 was also markedly suppressed in CCR6KO animals (Figure 1d). We have previously shown that γδ T cells account for the majority of production of IL-17A and IL-22 in the IL-23 PsD model (Mabuchi et al., 2011). Flow cytometry analysis of 710 consecutive clinically equivocal cases. J Invest Dermatol 2012;132: 2386–94.


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