High Frequency Acoustic Properties for Cutaneous Cell Carcinomas In Vitro

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Abstract—The present work studies the acoustic properties of cutaneous cell carcinomas for ex vivo tissue samples. An ultrasound biomicroscope, working at a central frequency of 45 MHz, was used. The analyzed parameters are sound speed, attenuation coefficient and attenuation coefficient slope. Additionally, normal tissues were used for comparison. Higher values of sound speed were observed in healthy skin, although not statistically different when compared with the carcinoma groups. Also, for the attenuation parameters no significant differences were evidenced. Presently, corrections in the methodology are being made for a more accurate characterization of carcinomatous skin.

Keywords—cutaneous carcinomas, ultrasound biomicroscopy, acoustic attenuation, sound speed.

I. INTRODUCTION

The standard method to diagnose dermatological lesions consists of tissue sample excision, and subsequent histological preparation for light microscopy visualization. This procedure can be undesirable in many situations, because of patient health condition or of aesthetic factors. Challenged by these limitations, several non-invasive imaging diagnostic techniques are being implemented in dermatology, to improve the patient healthcare protocol, as well as the health centers routine.

Ultrasound biomicroscopy (UBM) uses high-frequency acoustic waves for high-resolution images generation. The common frequency range for clinical applications is 20-60 MHz, giving image resolutions of few tens of micrometers, and penetration depths of few millimeters [1]. These conditions allow the differentiation of epidermal, dermal and subcutaneous layers, as well as the visualization of cutaneous annexes and anomalous structures.

Cutaneous carcinomas are the most common malignant tumors, and their principal predisposing factor is the sunlight exposure. They arise from malignant proliferation of epidermal and adnexal keratinocytes, and in the particular case of basal cell carcinomas (BCC), atypical basaloid cells nests are observed. This carcinomas are an important public health problem, despite their low mortality rate [2].

Several works have employed UBM imaging technique to study cutaneous carcinomas. Some of them were intended to measure tumor sizes [3; 4]; and in many of them the tumor sizes were overestimated because of unclear differentiation between tumor nests and the surrounding associated components. Other works were conducted for tumors regression evaluation after therapy, as well as for their recurrence incidence [5; 6]; in these works, UBM showed a great potential to characterize the neoplasm evolution. Moreover, some works studied the tumor echogenicity pattern as an indicator of specific carcinoma types; in this sense, Uhara et al. [7] related bright spots in UBM images of nodular and superficial carcinomas, with the presence of calcification foci, cornified cysts, cells clusters or necrosis; on the other side, Desai et al. [4] observed more echoic characteristics in morphoeiform BCC cases, due to a dense fibrous stroma surrounding the tumor nests.

The calculus of acoustic parameters was applied in the dermatology area, to identify spatial variations in normal skin, and in a less extent to analyze some anomalous conditions; conversely, studies of cutaneous carcinomas by acoustic parameters were not found in the literature. The commonly studied parameters are: wave speed (c), attenuation coefficient (α), attenuation coefficient slope (ηα), integrated attenuation coefficient (IAC), backscattering coefficient (β), backscattering coefficient slope (ηβ) and integrated backscattering coefficient (IBC).

Several studies were conducted to determine the spatial variability of acoustic parameters from healthy skin. Lebertre et al. [8] observed that IBC provides a good representation of skin structure variations as a function of dermis depth for tissues in vitro; additionally they showed that intra-individual variations of ηα, IAC and IBC were lower than the inter-individual ones. Raju and Srinivasan [9] studied the differences between ηα and β obtained for dermal and hypodermal tissues in vivo, and observed higher β-values in dermis, but no significant differences for ηα.

Few cases of anomalous skin conditions were analyzed by acoustic parameters. Miyasaka et al. [10] compared the c values for photo-damage and photo-protected areas, revealing higher speed in the papillary dermis of photo-damage areas, which was related to fibrosis presence. Huang et al. [11] measured ηα, IAC and IBC for in vivo dermis affected by radiation-induced fibrosis; in this case, smaller ηα values and larger IAC and IBC values were observed when compared to normal skin.

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In the present work it is evaluated the potential of acoustic parameters to characterize cutaneous carcinomas, using tissues \textit{ex vivo}. The analyzed tissues include several types of nodular BCC cases, as well as actinic keratoses (AK) and healthy skin for comparison. An UBM experimental system, operating with a center frequency ($f_c$) of 45 MHz, and capable to work as A and B-mode scanner was used. The measured acoustic parameters are $c$, $\alpha$ and $\eta$. Since there are considerable histological differences between healthy dermal collagen network and the tumor environment, a significant variation on their values is expected in advance.

II. MATERIAL AND METHODS

A. Tissue Samples

The present work was conducted with tissue samples \textit{ex vivo}, from the Dermatology Sector of the Gaffrée & Guinle University Hospital (HUGG) - Rio de Janeiro. The volunteer patients, under cutaneous carcinoma suspicion, were submitted to biopsy for diagnostic purposes. They were informed of the procedures and objectives of the present work, and agreed to participate.

Nineteen nodular BCC, four AK and four healthy skin tissue samples were studied. The last ones were obtained from the free-tumor border present in some biopsies. The AK samples were excised under carcinoma suspicion, and the carcinoma diagnostic was excluded by light microscopy analysis (Fig. 1). The nodular BCC cases were subdivided in accordance with the distribution patterns of tumor nests (Fig.1). The N1 group comprise numerous and small tumor nests, distributed along the dermis depth (ten samples); the N2 group consist of samples with the tumor nests growing in a circumscribed region (five samples); finally, the N3 group corresponds to ulcerated BCC cases (four samples).

The excised lesions were obtained from different body regions and were preserved in formalin solution after excision. Following UBM analysis, the biopsies were diagnosed by light microscopy in the Pathologic Anatomy Sector of the HUGG - Rio de Janeiro, and the results were also used for comparison with the ones by UBM.

All investigation procedures followed a protocol approved in accordance with the HUGG Ethical Committee and with the National Committee for Ethics in Research.

B. UBM System

An experimental system, working at 45 MHz, assembled at the Biomedical Engineering Program of the Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, was used for the images and RF signals acquisitions. Its principal characteristics are depicted in [12].

During acquisitions, the tissue sample was positioned over a sapphire disc (reflector material), inserted in a holder and covered by a polymer film; this assemblage was placed inside of an acrylic container filled with saline solution, which acted as a coupling medium between the tissue sample and the transducer surface.

![Fig. 1 BCC and AK images, obtained by UBM (left column) and light microscopy (right column). a-b) N1 group (see text); the tumor nests are not identified in UBM images which present heterogeneous characteristics. c-d) N2 group (see text), tumor nests demarked by points. e-f) N3 group (see text); the tumor nests are visualized like hypoechoic structures (right arrow) and the bleeding shows more echogenic characteristics (left arrow). g-h) AK images, showing hypoechoic aspect (right arrow); the epidermis detached from the dermis; the left arrow indicates a glandular structure.](image)

C. Acquisition Protocol

The region of interest (ROI) was firstly determined from B-mode images. It consists of a matrix of 8x8 points spaced by 50 $\mu$m, from where the RF signals were acquired.

The acoustic parameters were computed using the double transmission method [13]. It requires the collection of six signal groups (each group consisting in 64 RF signals acquired from all ROI points), with the transducer focus positioned at different depths of the tissue sample.