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Original research

Scanning acoustic microscopy imaging of tongue squamous cell carcinomas discriminates speed-of-sound between lesions and healthy regions in the mucous epithelium

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ABSTRACT

Objective: A scanning acoustic microscope can show images created from the high-frequency sound's speed through tissues. The objective of this study was to determine the availability of the scanning acoustic microscope in the diagnosis of the squamous cell carcinoma of the tongue.
Methods: We used SAM to obtain images of squamous cell carcinoma of the tongue, and we analyzed the differences in the sound speed between lesions and healthy regions in the mucous epithelium. The subjects were 10 patients (5 males and 5 females, 41–88 years old, avg. 64.0 years) who underwent partial resection of squamous cell carcinoma of the tongue.
Results: The results showed significantly higher sound speeds in the lesions compared to the healthy regions in the mucous epithelium. The scanning acoustic microscope provides the general benefits as follows: images are acquired in a few minutes without any staining and the sound speeds from each region are provided as digital data and comparable among diseases.
Conclusions: Our observations suggest that the scanning acoustic microscope imaging can be very helpful for determining the margin of squamous cell carcinoma.

1. Introduction

Visible images of body tissues and a determination of the hardness of such tissues can be obtained with a scanning acoustic microscope (SAM), which measures the differences in the speed of high-frequency sounds before and after the sound passes through a biological tissue. SAM has been used in clinical settings, but there have been very few reports of SAM examinations of the oral and maxillofacial region. In the present study, we used SAM to study one type of oral cancer, squamous cell carcinoma (SCC) of the tongue. We measured the speed of sound produced by a SAM system in the mucous epithelium of SCCs of the tongue, and we analyzed the measured values to determine the usefulness of SAM for delineating the tumor margin.

2. Materials and methods

2.1. Tissue samples

The subjects were 10 patients (5 males and 5 females, 41–88 years old, avg. 64.0 years) who underwent partial resection of an SCC of the tongue between December 2005 and February 2012 at the Departments of Oral and Maxillofacial Surgery, Hamamatsu Medical Center. We excluded patients who had been treated by chemotherapy and/or radiation therapy as a preoperative treatment, in order to exclude patients who had inflammation after preoperative treatment. The TNM classification of the primary site was T1 and T2 in all samples. The sample tissues were limited to well-differentiated SCCs and well-margined. The tissue samples were fixed overnight, in about 12 h, with 10% neutralized formalin, embedded in paraffin, cut at a thickness of 10 μm with a microtome at the suspected margins, and deparaffinized by alcohol and

☆ AsianAOMS: Asian Association of Oral and Maxillofacial Surgeons; ASOMP: Asian Society of Oral and Maxillofacial Pathology; JSOP: Japanese Society of Oral Pathology; JSOMS: Japanese Society of Oral and Maxillofacial Surgeons; JSOM: Japanese Society of Oral Medicine; JAMI: Japanese Academy of Maxillofacial Implants.

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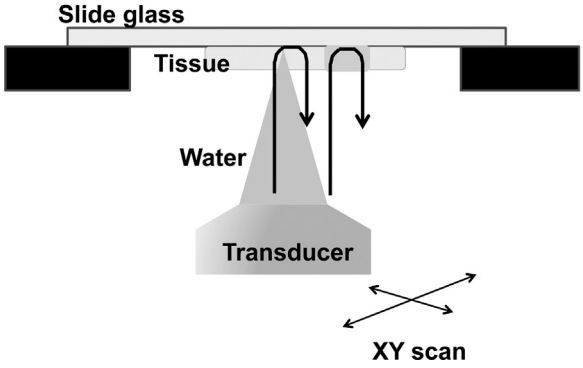

Fig. 1. The operating principles of scanning acoustic microscopy. The ultrasound emitted from the transducer hits the glass slide's surface and penetrates the tissue and returns to the transducer. The speed of the ultrasound moving through the tissue slice with distilled water is calculated by comparing the flight time of the pulse from the surfaces of both the tissue and the glass.

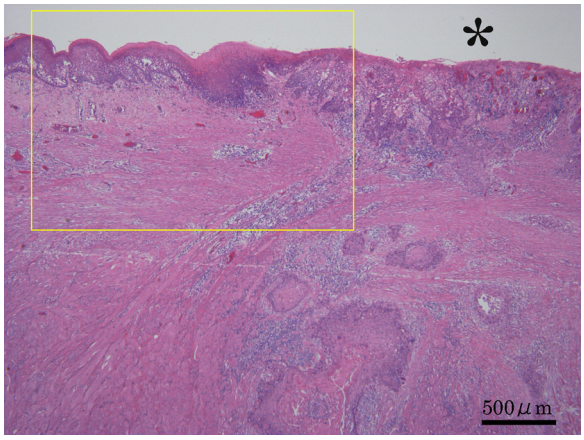

Fig. 2. Histopathology of case No. 2 (HE stain). Photomicrograph showing the cut end of the SCC of case No. 2. Asterisks indicate the tumor site. We observed a square area with SAM (see Fig. 3). The scale bar = 500 μm.

xylene. The tissue sections were mounted on glass slides without cover slips. No staining was needed for measurement with SAM.

2.2. Histologic staining

Hematoxylin-eosin (HE) staining was used to assess the overall histologic structure. Photographs were taken under the light microscope, and these were cropped to form histologic images with areas identical to those of the SAM measurements. Theoretically, the same tissue can be re-stained by HE after SAM examination, and be observed by the routine microscopy. However, in the present study, we did not do so. It was impossible to make HE staining immediately after SAM examinations, since the SAM was in the different institute (in Hamamatsu University, School of Medicine). Therefore, we compared the adjacent slices in the SAM and HE examinations.

2.3. Observation with SAM imaging for tissue characterization

We used SAM made by Honda Electronics Co. (Toyohashi, Japan), which has a resolution of approximately 13 μm (Fig. 1). SAM works as follows: focused high-frequency ultrasound is issued from a transducer toward the tissue on a glass slide. The sound hits the glass slide and penetrates the tissue on the slide, and the sound is reflected back to the transducer. The sound corresponds to a 120-MHz transducer after the sound is returned to the detector. A reflected wave is formed from the reflection at the front and rear surfaces of the tissue slice with distilled water between the transducer and section. Additionally, the attenuation and phase spectra are compared with those of the direct reflection at the glass surface where no tissue is present. The sound speed is calculated by comparing the time of flight of the pulse from the surface of both the tissue and the glass slide after mechanical X-Y scanning.

On the stage of SAM, we can adjust the location of the slide, the scan size (region of interest: 1.2 mm × 1.2 mm, 2.4 mm × 2.4 mm, or 4.8 mm × 4.8 mm), and/or the scan points (maximum, 300 × 300 points). The time of scanning depends on the number of scan points. At the same number of scan points, to get the higher resolution, the smaller scan size is needed. In the present study, we scanned the slide at 300 × 300 points in the 2.4 mm × 2.4 mm area. The single scan required about 2 min. We obtained two-dimensional profiles of reflection intensity and created color-coded images, and thus we observed the sound speed of each lesion and healthy region in the mucous epithelium of ten SCCs.

3. Results

As shown in Fig. 2, we confirmed the boundary between the lesion and healthy region in the mucous epithelium of each of the SCCs by HE stain. We then depicted an intensity image in the non-stained area and compared it with the corresponding acoustic image (Fig. 3), measuring three points of the sound speed for each lesion and healthy region in the mucous epithelium with SAM. We observed the same results in the different represented cases (Fig. 4).

We calculated the average sound speeds for each of the ten SCCs (Table 1). The mean sound speed of the healthy regions in the mucous epithelium was 1771.077 m/s, and that of the lesions in the mucous epithelium was 1940.362 m/s, with a difference

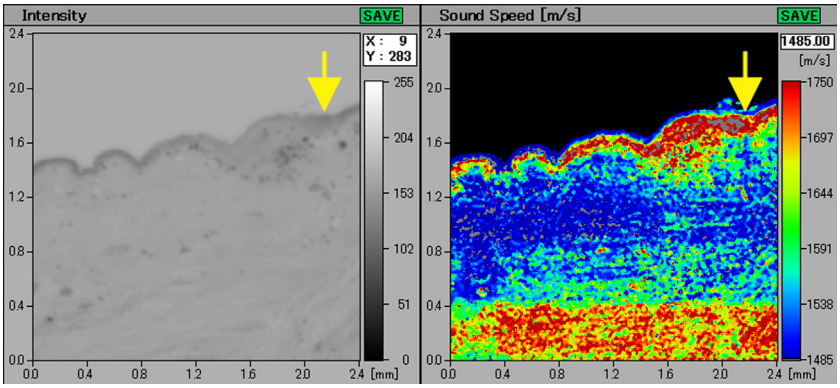

Fig. 3. Intensity image (left) and corresponding acoustic image (right) of case No. 2. The speed of sound through the lesion is faster than the speed through the healthy region in the mucous epithelium (arrow).