Open Journal of Stomatology, 2023, 13, 12-24
https://www.scirp.org/journal/ojst
ISSN Online: 2160-8717
ISSN Print: 2160-8709

Application of Scanning Acoustic Microscopy for Detection of Dental Caries Lesion

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How to cite this paper: Naganuma, Y., Hatori, K., Iikubo, M., Takahashi, M., Hagiwara, Y., Kobayashi, K., Takahashi, A., Hoshi, K., Saijo, Y. and Sasaki, K. (2023) Application of Scanning Acoustic Microscopy for Detection of Dental Caries Lesion. *Open Journal of Stomatology*, **13**, 12-24.
https://doi.org/10.4236/ojst.2023.131002

Received: November 25, 2022
Accepted: January 14, 2023
Published: January 17, 2023

Abstract

Introduction: A scanning acoustic microscope (SAM) is an apparatus for imaging acoustic properties. This apparatus can non-invasively and rapidly evaluate the hardness of materials in the elastic region. This device shows great potential for the diagnosis of dental caries in the clinical setting. However, since the tissue elastic modulus measured using a SAM is a property of the elastic region and the Knoop hardness is a property of the plastic region, the hardness properties differ completely. Therefore, we investigated whether the acoustic impedance measured using a SAM is related to the Knoop hardness, which is used as the standard for removal of carious dentin. **Method:** Polished sections were prepared from 20 extracted carious wisdom teeth. The acoustic impedance and Knoop hardness were measured for each section. In addition to comparing carious and healthy dentin in SAM images, we evaluated the difference between the carious and healthy dentin in terms of the acoustic impedance and Knoop hardness. We also evaluated the correlation between the Knoop hardness and acoustic impedance. **Results:** The SAM images were visualized as two-dimensional color images based on the acoustic impedance values. The mean acoustic impedance of carious dentin was significantly lower than that of healthy dentin, showing a similar trend as Knoop hardness. A strong correlation was observed between the two. **Discussion:** The acoustic impedance values obtained through acoustic microscopy differed significantly between carious and sound dentin. Both types of dentins were visualized using two-dimensional color images. A strong correlation was observed between the acoustic impedance value, which indicates the hardness of the elastic region, and the Knoop hardness, which indicates the hardness of the plastic region. The results of the present study indicate that acoustic impedance accurately reflects the hardness of dentin.

Keywords

Scanning Acoustic Microscope, Acoustic Impedance, Caries, Knoop Hardness

1. Introduction

Dental caries and periodontal disease constitute the two major diseases in dentistry. Dental caries is frequently diagnosed and treated in routine dental practice. Minimal intervention (MI) is crucial during the treatment of dental caries to ensure that only the carious dentin is removed [1]. Based on in vitro histological experiments using an optical microscope, Sano *et al.* [2] reported that the Knoop hardness number (KHN) is less than 20 in regions infected by bacteria in active carious lesions. The guidelines from the Japanese Society of Conservative Dentistry [3] for the treatment of dental caries stipulate that carious dentin with a KHN of less than 20 should be removed.

Steel round burs and hand excavators are primarily used to remove softened carious dentin [4] [5]. However, it is impossible to remove softened carious dentin while measuring the Knoop hardness, and the evaluation of the hardness of carious dentin relies on the dentist's subjective touch.

In 1973, an acidic red propylene glycol solution (caries detector®; Novadent, Lod, Israel) was introduced as an aid in identifying caries-affected regions [6]. However, this technology based on dye application helps in determining the cutting range based on the surgeon's visual observation; therefore, it is not an objective index [7]. If a device that can objectively evaluate the softened carious dentin depending on the hardness is developed, it will be possible to remove only the carious portion and ensure MI, which will improve the outcomes of caries treatment.

If hardness is evaluated as a valid criterion for caries removal, it is conceivable to apply a scanning acoustic microscope (SAM), which quantitatively measures the acoustic properties of materials, for the diagnosis of caries. A SAM is a device that emits ultrasonic waves toward an object to be observed through a medium, such as water, and captures the acoustic properties obtained by analyzing the reflected or transmitted waves in a minute area. Since the obtained acoustic parameters have a constant relationship with the tissue elastic modulus, which indicates the hardness of the elastic region of the material, the biophysical properties of the material can be measured using a SAM. Furthermore, it has the advantage of possessing the ability to observe tissues in real-time non-invasively and without staining [8] [9] [10] [11]. This device is primarily used in medicine for diagnosing diseases of the soft tissues of the body, such as the cardiovascular system and articular cartilage. Clinical applications are evolving with each passing day, and it is now possible to understand pathologies using 3D color imaging [12] [13]. Remarkable advancements have been seen in recent years, such as miniaturization of peripheral devices and development of oscillators suitable for various applications [14].

The ultrasonic caries detector® (hereinafter UCD; Novadent) was developed in 2003 as a device to aid in the diagnosis of caries using the principle of a SAM. This device measures the acoustic reflection emitted by the tooth surface by applying the transducer to the tooth surface; the signal is temporarily displayed on the screen. Matalon *et al.* [15] [16] investigated the reliability of diagnosing proximal caries using UCD compared with bitewing X-ray examination in vitro and in vivo. They reported high sensitivity and specificity and stated that ultrasound provides a new testing modality that can reduce exposure to ionizing radiation, possibly improving caries detection. However, this device can only determine the presence or absence of caries, not the extent of caries.

Therefore, we attempted to discriminate carious dentin from sound dentin by quantitatively evaluating the acoustic characteristics of dentin around cavities in our previous study, with the aim of examining the possibility of applying acoustic microscopy to the diagnosis of dental caries. A clear difference was noted in the acoustic characteristics between carious and sound dentin, and it was possible to depict the extent of caries [17] [18].

However, since the tissue elastic modulus measured with a SAM is a property of the elastic region, whereas the Knoop hardness is a property of the plastic region, the hardness properties differed completely. To the best of our knowledge, no studies in the literature have clearly reported a correlation between hardness and tissue elastic modulus. Therefore, the use of the results of acoustic properties alone to determine the extent of caries is not recommended. In this experiment, we investigated whether the acoustic impedance value measured using a SAM is related to the Knoop hardness, which is used as the standard for removal of carious dentin, to determine if the results of the measurement of acoustic properties using a SAM have clinical significance.

2. Samples and Method

2.1. Preparation of Samples (Carious Teeth)

The inclusion criteria were as follows: patients who visited the Tohoku University Graduate School of Dentistry and underwent extraction of their wisdom teeth, as tooth conservation was deemed impossible due to pericoronitis of the wisdom tooth or caries. These patients were briefed about the purpose of our study. Extracted wisdom teeth were collected from patients who provided consent. Among the extracted teeth, 20 teeth with cavities on one adjacent tooth surface were used as samples for this experiment upon visual examination. The exclusion criteria were as follows: 1) the patient or patient's family refuses to donate the extracted tooth and 2) the extracted tooth had significantly damaged crown morphology.

This study was approved by the Ethics Review Board of the Tohoku University Graduate School of Dentistry (reference number: 2019-3-037). All participants were fully informed about the study procedures and their right to withdraw from the study without any consequences. All participants provided written informed consent prior to participating in the study. All experiments on human subjects were conducted in accordance with the Declaration of Helsinki (http://www.wma.net).

The extracted teeth were embedded in a room-temperature polymerizing resin (SPLINT RETAINER RESIN; GC, Tokyo, Japan) and sliced to sections of 500-μm thickness in the sagittal direction using a wheel saw (Low-Speed Diamond Wheel Saw; South Bay Technology, San Clemente, CA, USA). After marking measurement landmarks on the sections, mechanical polishing (S5629; Marumoto Struers, Tokyo, Japan) was performed in combination with ultrasonic cleaning to prepare polished sections. Macro images of the polished sections were taken (EZ4HD; Leica microsystems, Wetzlar, Germany) subsequently, and the region where the brown-to-black coloring was visually observed in the cavity was regarded as the carious site (carious dentin) in this experiment.

2.2. Observation Using the Scanning Acoustic Microscope

2.2.1. Scanning Acoustic Microscope System

A SAM (AMS-50SI, Honda Electronics, Aichi, Japan) was used for this experiment. The overall view and block diagram of the SAM apparatus are shown in Figure 1 and Figure 2, respectively. The SAM performs two-dimensional scanning with an ultrasonic transducer and non-invasively measures the acoustic parameters of tissue sections and cells at the micro level. The information obtained is visualized on the display as a clear image. The microscope comprises five units. In this experiment, with reference to our previous research [17] [18], the pulse generator was set to a voltage of 40 V, a rise time of 400 ps, and a pulse width of 2.0 ns. The transducer used had a central frequency of 80 MHz, an

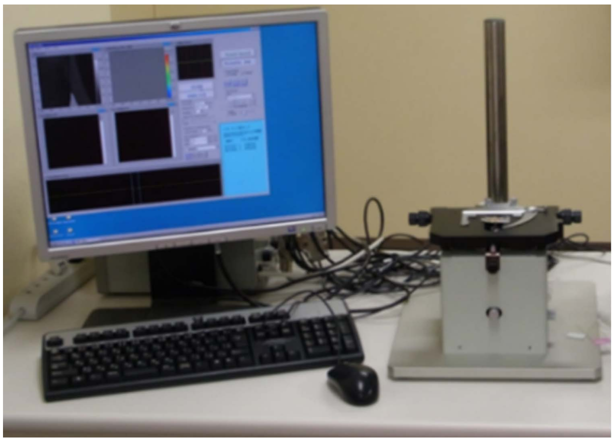

Figure 1. Scanning acoustic microscope (SAM).

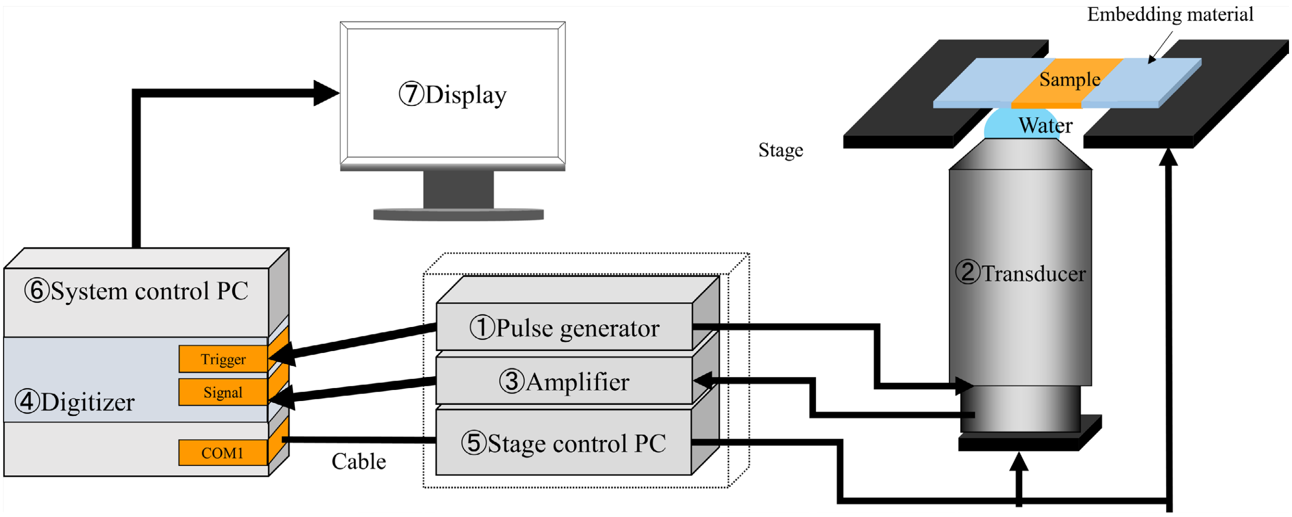

Figure 2. Block diagram of the scanning acoustic microscope.

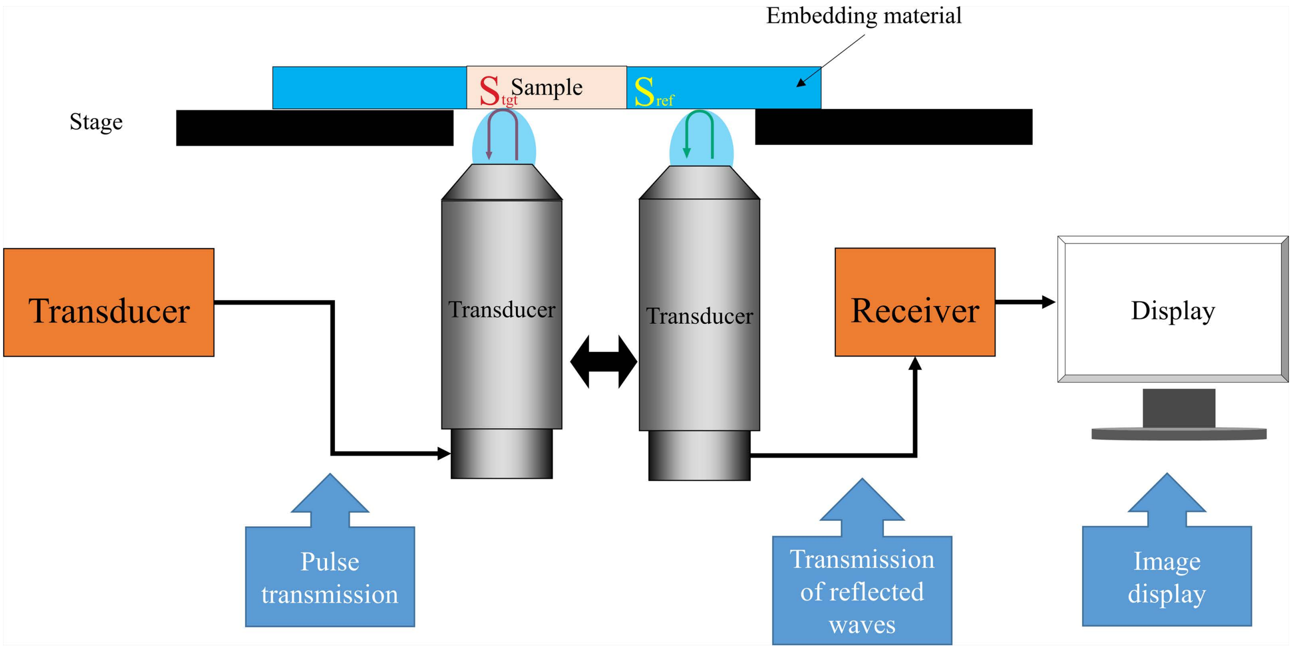

Figure 3. Expanded view of the measuring unit.

aperture of 1.8 mm, and a focal length of 2.5 mm. Figure 3 shows the expanded view of the measuring unit. S_{tgt} is the reflected wave from the sample, and S_{ref} is the reflected wave component from the embedding medium. The ultrasonic waves are emitted and received by the same transducer.

The scanning acoustic microscope mainly consists of five units: 1) ultrasonic transducer with acoustic lens, 2) pulse generator, 3) digital oscilloscope with system control PC (Windows), 4) stage control microcomputer and 5) display monitor

Ultrasound waves were emitted from the transducer. The three reflections from the glass surface, the sample surface, and the interface between the sample and the glass were received by the same transducer. The data from the three reflections were introduced into signal processing to visualize the SAM image.

2.2.2. Acoustic Impedance Measurement and Imaging

We observed the carious and sound dentin using the SAM. The observation range was 4.8 mm × 4.8 mm per measurement. The acoustic impedance data of each pixel was sampled at 1.0 GHz digitizer card (Agilent, U1065A; Santa Clara, CA, USA) and transferred to the system control PC in batches. The acoustic impedance values obtained were divided into 256 color tones within the range of 2.5 kg/m^{2}s (blue) to 10.0 kg/m^{2}s (red) and displayed as color images on the display.

The acoustic impedance values of the carious and sound dentin were measured in 10 points of each sample, and the mean value was regarded as the acoustic impedance value of the measurement site.

2.3. Knoop Hardness Measurement

The Knoop hardness for each sample was measured at the site where the acoustic impedance was measured with the SAM. The Knoop hardness was measured using a microhardness tester (HM-221; Mitutoyo, Kanagawa, Japan) equipped with a Knoop indenter, and the relationship with acoustic impedance was investigated. Based on the previous study by Shimizu *et al.*, the experimental conditions were a load of 0.245 N (25 gf) and a holding time of 15 seconds [19] [20].

2.4. Statistical Analysis

The Shapiro-Wilk test was used to analyze whether the Knoop hardness and acoustic impedance values were normally distributed. Since both were confirmed to be normally distributed, the Levene test was performed to confirm homoscedasticity. Since no homoscedasticity was observed, Welch's t-test was used to investigate whether a difference was present between the carious and sound dentin.

In addition, Pearson's correlation analysis was used to analyze whether a correlation was present between the Knoop hardness and the acoustic impedance values. The significance level for all tests was set at 0.05.

3. Results

3.1. Images Captured by the Scanning Acoustic Microscope

Figure 4 shows the SAM images of the carious dentin and macro images of the same site. The boundary line of the region shown in blue on the SAM image matched the dark brown region deemed to be carious dentin in the macro-image. Acoustic impedance images were visualized as two-dimensional color images, and the color images of sound and carious dentin were distinctly different. The reference embedding resin is shown in light blue, and the hollow region of the sample is shown in deep blue as it is filled with water. The acoustic impedance values in the enamel exceeded 10 kg/m^{2}s; thus, it is shown in red. The carious dentin tended to become darker blue toward the surface layer. The area defined as sound dentin in the macro-image is shown in green to yellowish green.

Ultrasound waves were emitted from the transducer. The three reflections from the glass surface, the sample surface, and the interface between the sample and the glass were received by the same transducer. The data from the three reflections were introduced into signal processing to visualize the SAM image.

3.2. Comparison of the Knoop Hardness and Acoustic Impedance in Carious and Sound Dentin

Figure 5 presents a comparison of the Knoop hardness in carious and sound dentin. The mean Knoop hardness in carious dentin (12.15 ± 4.47 kg/m²s) was significantly lower than that in sound dentin (62.79 ± 4.10 kg/m²s) ($p < 0.01$).

Figure 6 presents a comparison of the acoustic impedance in carious and sound dentin. The mean acoustic impedance in carious dentin (3.62 ± 1.29 kg/m²s)

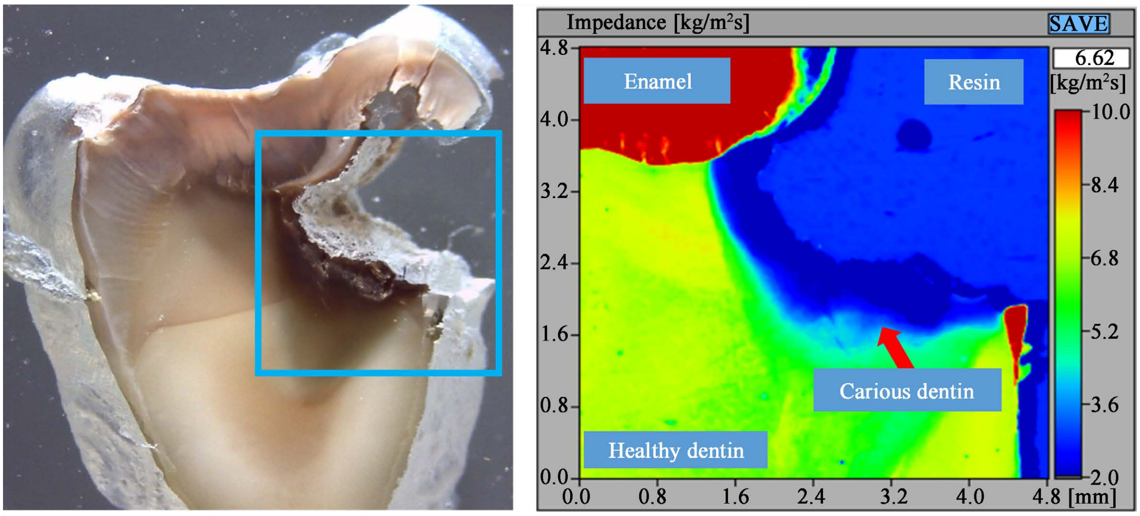

Figure 4. Comparison of the macroscopic image and scanning acoustic microscope image of the section.

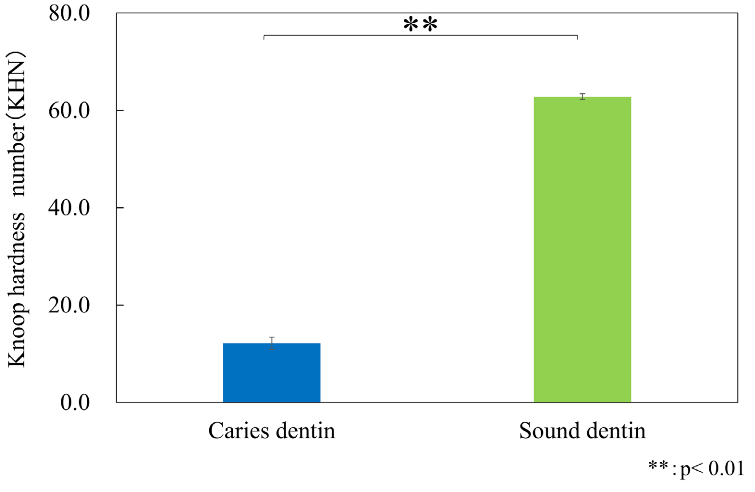

Figure 5. Comparison of Knoop hardness.

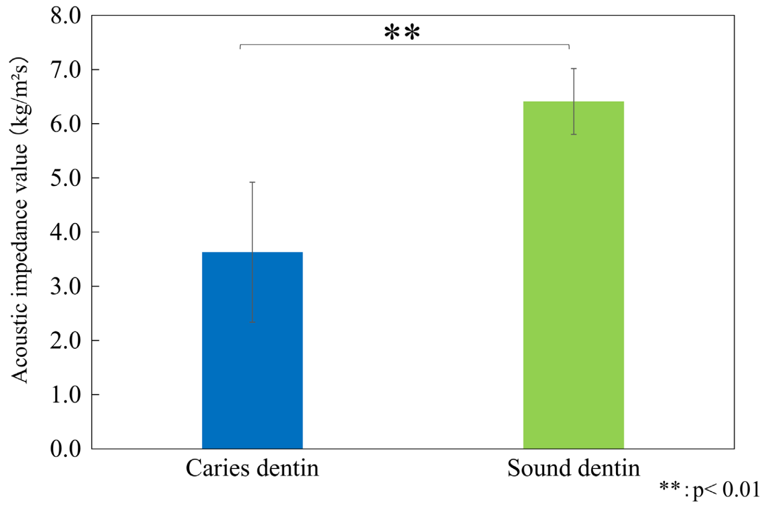

Figure 6. Comparison of acoustic impedance.

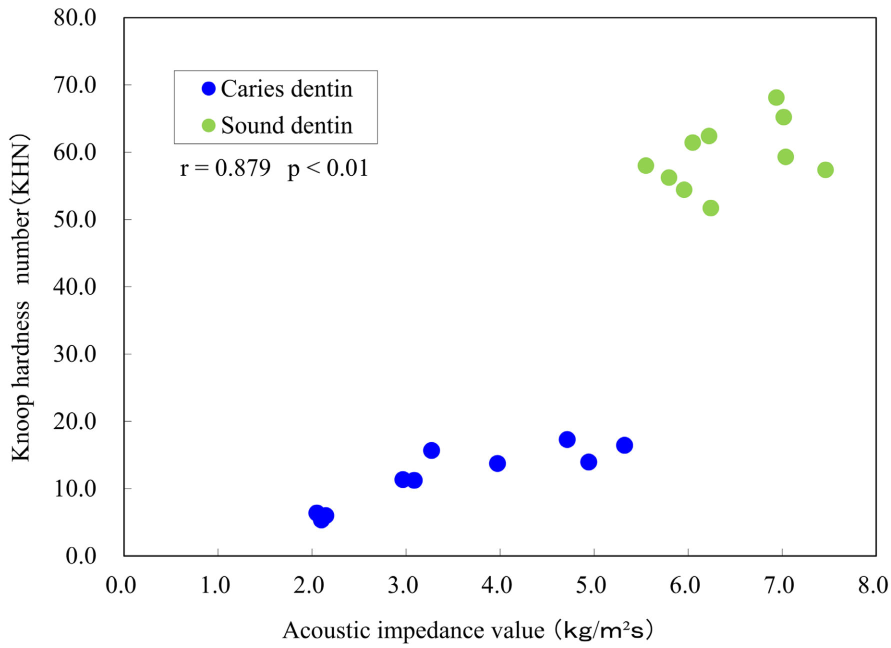

Figure 7. Relationship between acoustic impedance and Knoop hardness.

was significantly lower than that in sound dentin (6.48 ± 0.61 kg/m²s) ($p < 0.01$).

Figure 7 shows a correlation plot of the acoustic impedance and Knoop hardness. A strong positive correlation was identified between the two ($r = 0.879$; $p < 0.01$).

4. Discussion

Dental caries is the most prevalent disease in dentistry. According to the World Health Organization (WHO) report in 2015, approximately 60% - 90% of adults and nearly 100% of children have dental caries [21]. Therefore, accurate diagnosis of dental caries is of utmost importance in dentistry. However, only few objective investigation methods are currently available to detect dental caries.

Histological diagnosis by acoustic microscopy depends on visualization based on acoustic parameters. Acoustic microscopy can be used to evaluate elastic properties; however, it has been used primarily for soft tissue, as the reflection of signals makes its use unsuitable for hard tissue [22]. Despite only being an observation of sections of extracted teeth, our study was able to visually distinguish carious and sound dentin via images with distinctly different colors using a SAM.

This visualization is based on the acoustic impedance values measured using the SAM. Acoustic impedance (z), sound velocity I, density (ρ), and tissue elastic modulus (κ) have the following relationship $\kappa = \rho c^2 = z^2/\rho$; therefore, the changes in acoustic parameters can objectively ascertain the tissue elastic modulus, *i.e.*, the change in hardness of a substance [22] [23]. The elastic properties of tissue reflect pathological changes. Protozoan bacteria destroy the structure of sound dentin and reduce its hardness and elastic modulus [19]. Our experiments also showed that carious dentin had lower Knoop hardness and acoustic impedance than sound dentin.

Histological diagnosis using a SAM has been primarily applied to soft tissue as it does not require thin sections or staining and can generate an acoustic color image [8] [24]-[32]. While previous studies have investigated its application in dentistry [15] [16] [33] [34] and it is possible to measure the inside of hard tissue non-invasively using ultrasound, no diagnostic equipment for dental caries has been developed. Since enamel has high acoustic impedance values, it is impossible to take two-dimensional color images. However, this study has demonstrated that it is possible to evaluate the elastic properties of teeth by determining the acoustic impedance using a SAM, and sound and carious dentin on the sample surface can be visualized as a two-dimensional color image. The hardness estimated using the SAM is the hardness in the elastic region based on the bulk modulus that varies within the proportional limit. However, since the Knoop hardness is the hardness in the plastic region measured using a breaking test, the hardness properties are entirely different. Therefore, it was essential to determine whether the result of assessing caries using a SAM was accurate.

In the SAM images obtained in our experiment, the sound dentin was shown in green to yellow, corresponding to the acoustic impedance values of 6.0 - 8.0 kg/m^{2}s, and the mean acoustic impedance values were also within this range. Therefore, it was presumed that the green-yellow region had a KHN of more than 60. The acoustic impedance values of carious dentin ranged from 2.04 - 3.23 kg/m^{2}s, and most of this area was dark blue. The results showed that the acoustic impedance of carious dentin lesions was significantly lower than that of sound dentin, and a strong correlation between the Knoop hardness and acoustic impedance values was observed. Another way of describing this result is that there is a high correlation between the Knoop hardness and the bulk modulus. Since the correlation between the elastic region's hardness and the plastic region's hardness was significantly high, it can be assumed that this device could accurately identify carious dentin.

The results of this study show that, unlike the acoustic caries detectors developed in the past [15] [16], our device can detect the spread of caries and provide beneficial information for the diagnosis and treatment of caries.

Based on these findings, we have shown that the acoustic impedance values measured using the SAM can provide vital information for diagnosing and treating caries.

In the future, we plan to develop ultrasonic caries testing equipment that uses acoustic impedance values for the oral cavity, based on the data obtained in this study, to establish a diagnostic method for caries that is high in quality, uniform, and independent of the subjective assessment and skill level of the dentist.

5. Conclusion

We observed thin sections of extracted human teeth with caries using a SAM and succeeded in obtaining color images based on the acoustic impedance values. The acoustic impedance values of carious dentin were significantly lower than those of sound dentin, and the resulting SAM images showed carious dentin and sound dentin in distinctly different colors, suggesting the possibility of applying acoustic microscopy to the diagnosis of caries.

Acknowledgements

This work was supported by JSPS KAKENHI Grant Number JP19K190640.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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