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Effect of Dentin Hardness on Ablation Rate with Er:YAG Laser

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Abstract

Objective: This study used artificially demineralized bovine dentin to ascertain the effect of hardness of demineralized dentin on ablation rate with the Er:YAG laser. *Background Data:* Before restoration of carious teeth, it is necessary to ablate any infected dentin that cannot be remineralized due to softening by the invading caries. A correlation has been suggested between the ablation efficiency of the Er:YAG laser and the progression of caries in dentin. To the best of our knowledge, no studies have investigated the relationship between degree of demineralization by dentinal caries and ablation rate with the Er:YAG laser. *Materials and Methods:* Bovine mandibular anterior tooth roots were used as dentin samples. Each sample was soaked in a demineralizing solution (2 M lactic acid, pH 4.0) for 3 d to obtain demineralized dentin (DD) samples. Another group of samples were prepared without demineralization as a sound dentin (SD) group for comparison. After determining the Knoop hardness number (KHN) of each sample, the dentin surface was ablated with an Er:YAG laser. Tip output and pulse rate were set at 50 mJ and 20 pulses per second (pps), respectively, and the water spray was set at 3.5 mL/min. Ablation width, depth, and volume were measured following irradiation. *Results:* In the DD group, dentin hardness was 10.4 ± 1.6 KHN at 100 μm from the surface. In the sound dentin group, Knoop hardness was 51.0 ± 1.6 KHN cross-sectionally throughout. No differences were observed from the surface to a depth of 2000 μm. In the DD group, dentin ablation volume at the superficial demineralized layer was $2888 \pm 272 \times 10^4$ μm. In the SD group, dentin ablation volume was 1298 ± 219 μm^3. The relationship between ablation volume and Knoop hardness was defined as $Y = -40.699x + 3350$, revealing a marked negative correlation. *Conclusion:* The results demonstrate that the ablation volume for demineralized dentin was greater than that for sound dentin. The results suggest that the Er:YAG laser is capable of ablating infected dentin without damaging sound dentin by adjustment of tip output and pulse rate.

Introduction

INFECTED DENTIN can be explained by Miller's chemoparasitic theory, in which minerals are removed by acids produced by caries-inducing bacteria, and organic compounds are decomposed by collagenase. In moderate caries, bacteria are only found in areas with a hardness of <20 KHN (Knoop hardness number).[1]

In recent years, use of the Er:YAG laser has become widespread, as it allows ablation of hard tissue. Various studies have investigated Er:YAG laser treatment for caries in terms of pulp safety, restoration compatibility, water spray, output power, and pulse rate.[2–5] However, most of these studies used healthy teeth, and studies on carious teeth are lacking.

Compared to other types of laser, the wavelength of the Er:YAG laser has the highest absorption rate by water. Effective ablation of biological tissues with infrared lasers is largely dependent on the presence of water.[6] As infrared radiation couples with water molecules, it causes an increase in the vibration of those molecules, increasing temperature and pressure within the tissue. Ablation is initiated as an explosive thermal event. Based on the strong reaction that the Er:YAG laser elicits in water molecules, it was hypothesized that a correlation might exist between ablation rate and degree of demineralization in dentin.

To the best of our knowledge, no studies have investigated the relationship between degree of demineralization by dentinal caries and ablation rate with the Er:YAG laser. This

study utilized artificially demineralized bovine dentin to ascertain the effect of hardness of demineralized dentin on ablation rate with the Er:YAG laser.

Materials and Methods

Preparation of dentin plates

Extracted and frozen bovine mandibular anterior tooth roots were thawed for use just prior to commencement of the study. To ensure uniformity of direction in the dentinal tubules, root dentin cut at the cementoenamel junction was split labiolingually, and the buccal-side surfaces were used. Sectioned surfaces were polished using water-resistant abrasive paper of 220–1200 grit, after which the other unpolished surfaces were covered with three coats of nail varnish, so that the demineralizing solution would only come into contact with the polished surfaces (Fig. 1). Each sample was demineralized with 2 M lactic acid (pH 4.0) for 3 d at room temperature, and the surfaces of the samples were washed and soaked in distilled water for 1 d. Each sample was then split vertically along the tooth axis and embedded in epoxy resin (Scandiprex, Fritsch, Japan, Tokyo, Japan).

After hardening of the embedded resin, the surfaces to be measured were exposed and polished using water-resistant abrasive paper of 800–1200 grit, followed by mirror polishing using alumina (grain size: 0.05 μm) (DD group). For comparison, non-demineralized samples (sound dentin; SD group) were prepared in the same manner, but without soaking in demineralizing solution.

One half of each vertically split sample was used for measurement of hardness. The other half was used for measurement of ablation volume. The measuring surfaces of the DD and SD groups are shown in Fig. 2. A total of 13 samples were prepared, comprising 8 DD samples and 5 SD samples. Each measuring surface was divided into two areas, the medial and the distal area of the pulp cavity, yielding a total of 26 surfaces, comprising 16 DD surfaces and 10 SD surfaces.

Measurement of hardness

The Knoop hardness of the mirror-polished samples was determined with a micro-hardness tester (HMV1; Shimadzu, Tokyo, Japan). In each sample, hardness was measured at 100-μm intervals up to 2000 μm from the surface to the lateral side. Load was set at 1 N at the demineralized area and 3 N at the normal area, and holding time was set at 15 sec.

Ablation using Er:YAG laser

Laser device. The Er:YAG laser device used in this study was the Adverl (Morita Co., Tokyo, Japan), emitting a
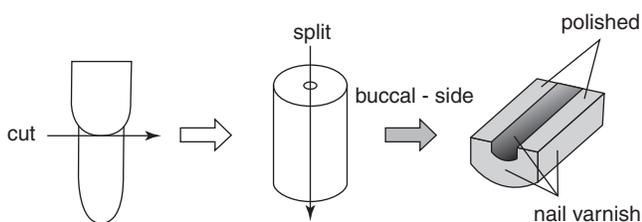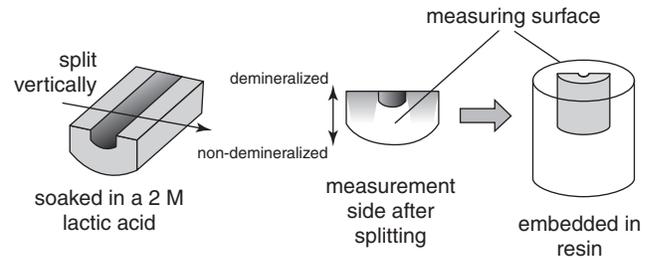

FIG. 2. Measuring surfaces for demineralized dentin and sound dentin groups.

wavelength of 2.94 μm. It has a flexible fiberoptic guide, a quartz contact chip, and a cooling system that operates by mixing air and water that is sprayed from the handpiece during laser irradiation. The Er:YAG laser had an 800-μm tip (C800F hard-tissue contact probe; Morita Co.). This laser can provide an output energy of 30–350 mJ/pulse, and a pulse repetition rate of 1–25 pulses per second (pps).

Irradiation methods and conditions. To achieve continuous irradiation, a mobile platform (S-46 Controller; Chuo Seiki, Tokyo, Japan) was used. The mobile platform was fixed so that the center of the sample was perpendicular to the laser beam, and the distance from the dentin surface to the tip was fixed at 0.05 mm using a height gauge (Hutaba,Tokyo, Japan) to minimize energy attenuation due to distance and scattering by the water spray. Velocity was set at 1 mm/sec, so that the Er:YAG laser could sufficiently irradiate the dentin within a clinically applicable range. Tip output and pulse rate were set at 50 mJ and 20 pps, respectively, so that dentin in healthy areas could be ablated without over-ablation of demineralized areas. Volume of water spray was set at 3.5 mL/min, and energy density was 208.3 J/cm^2.

Measurement of ablation width, depth, and volume. Ablation width, depth, and volume following irradiation were measured using a VK-8500 3D-color laser microscope with a He-Ne laser (VK-8500; Keyence, Osaka, Japan). The reflecting light of the He-Ne laser was measured on an image on a TV monitor using a CCD camera. The accuracy of measurement was 0.01–0.03 μm. After measurement, cross-sectional images of the ablated areas were prepared, and ablation width was defined as an imaginary line between both ends of the superficial laser-induced transpiration layer. Based on these measurements, three-dimensional images were constructed. Ablation volume was calculated from the superficial layer in 100-μm increments to obtain ablation rate.

Statistical analyses

Knoop hardness and ablation volume at each depth from the superficial layer were analyzed using the Scheffe test, with the level of significance set at $p < 0.05$. The relationship between Knoop hardness and ablation volume was statistically assessed in further detail using Spearman's correlation coefficient with the significance level set at $p < 0.05$.
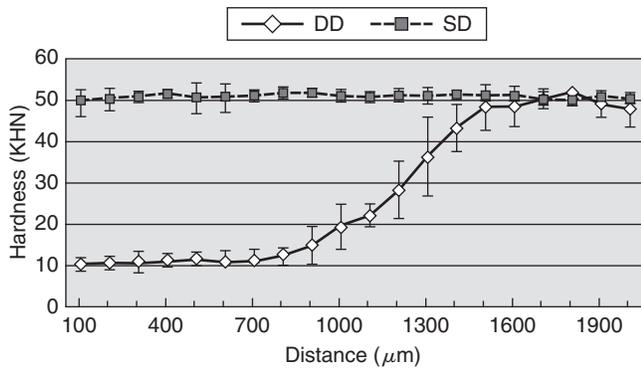

FIG. 1. Sample preparation method.

FIG. 3. Relationship between Knoop hardness and distance from the surface.

Results

Dentin hardness

The relationship between Knoop hardness and distance from the superficial demineralized layer was established (Fig. 3). Dentin hardness was lowest in the DD group (10.4 ± 1.6 KHN) at a distance of 100 μm, it increased to 900 μm, and peaked (51.0 ± 1.6 KHN) at 1800 μm. No differences in Knoop hardness were observed in the SD group from the surface area to a distance of 2000 μm (51.0 ± 1.6 KHN).

Table 1 shows the results of the statistical comparison between the SD and DD groups. Compared to the SD group, Knoop hardness near the superficial demineralized layer was significantly lower in the DD group. However, at distances beyond 1500 μm, no significant differences in Knoop hardness were observed between the DD and SD groups. In the DD group, Knoop hardness was >20 KHN (22.2 KHN) at 1100 μm, and 48.3 ± 5.54 KHN at 1500 μm.

Ablation volume

Fig. 4 shows ablation width and ablation depth at 500, 1300, and 1800 μm from the measuring surfaces.

Fig. 5 shows the relationship between ablation volume and depth points from the superficial layer. Dentin ablation volume at the superficial demineralized layer was 2888 ± 272 × 10^4 μm³, decreased around 700 μm, and reached 1165 ± 293 × 10^4 μm³ at 1900 μm.

Fig. 6 shows ablation volume and Knoop hardness at each measurement point. The relationship between ablation volume and Knoop hardness was defined as y = −40.699x + 3350, yielding a markedly negative correlation, with a correlation coefficient of r^2 = 0.98.

Discussion

Tooth hardness can be measured using a micro-hardness tester such as the Vickers or Knoop hardness testers, or a nano-indentation tester. The hardness of complex tissues, including peritubular dentin, intertubular dentin, and dentinal tubules, varies depending on location, so the hardness of deep dentin with dense dentinal tubules is lower than that seen in more superficial layers.[7] The hardness of human dentin was found to decrease from the cementodentinal junction towards the pulp cavity.[8] No significant differences in Knoop hardness in the SD samples were identified in relation to the points measured in this study. The load of the Knoop hardness tester was set at 1 N and 3 N, so that the indentation size was large enough to cover the peritubular dentin and dentinal tubules. Measurements were thus unaffected by peritubular dentin or dentinal tubules.

Regarding the hardness and clinical significance of carious dentin, Paolinelis et al. reported that bacteria were only found in areas with a hardness <20 KHN.[1] Hossain et al. reported that the hardness of residual dentin was about 20 KHN after ablating softened dentin as much as possible using a spoon excavator.[9] In this study, Knoop hardness was <20 KHN from the superficial layer to a distance of 1000 μm deep in the DD group. Therefore, we believe that the samples used here were appropriate for investigating the relationship between hardness and ablation volume.

The first layer of carious dentin was selected for ablation: demineralized dentin with a hardness of <20 KHN to a depth of 1000 μm.

TABLE 1. STATISTICAL ANALYSES OF KNOOP HARDNESS OF THE DD AND SD GROUPS

Depth	(μm)	100	200	300	400	500
SD	(KHN)	49.5 ⌐	50.3 ⌐	50.9 ⌐	51.5 ⌐	50.5 ⌐
DD	(KHN)	10.4 ⌐*	10.6 ⌐*	10.9 ⌐*	10.9 ⌐*	11.5 ⌐*
Depth	(μm)	600	700	800	900	1000
SD	(KHN)	50.7 ⌐	51.1 ⌐	51.9 ⌐	51.7 ⌐	51.3 ⌐
DD	(KHN)	10.8 ⌐*	11.2 ⌐*	12.2 ⌐*	15.1 ⌐*	19.5 ⌐*
Depth	(μm)	1100	1200	1300	1400	1500
SD	(KHN)	51.2 ⌐	51.3 ⌐	51.3 ⌐	51.6 ⌐	51.4
DD	(KHN)	22.2 ⌐*	28.3 ⌐*	36.4 ⌐*	43.2 ⌐*	48.3 *
Depth	(μm)	1600	1700	1800	1900	2000
SD	(KHN)	51.4	51.0	50.8	51.1	50.5
DD	(KHN)	48.6 *	50.6 *	51.8 *	49.2 *	47.9 *

*p < 0.05.

demineralized distance from the
measuring surfaces (μm)

222.73
μm

1800

1489.999 [μm]

242.27
μm

1300

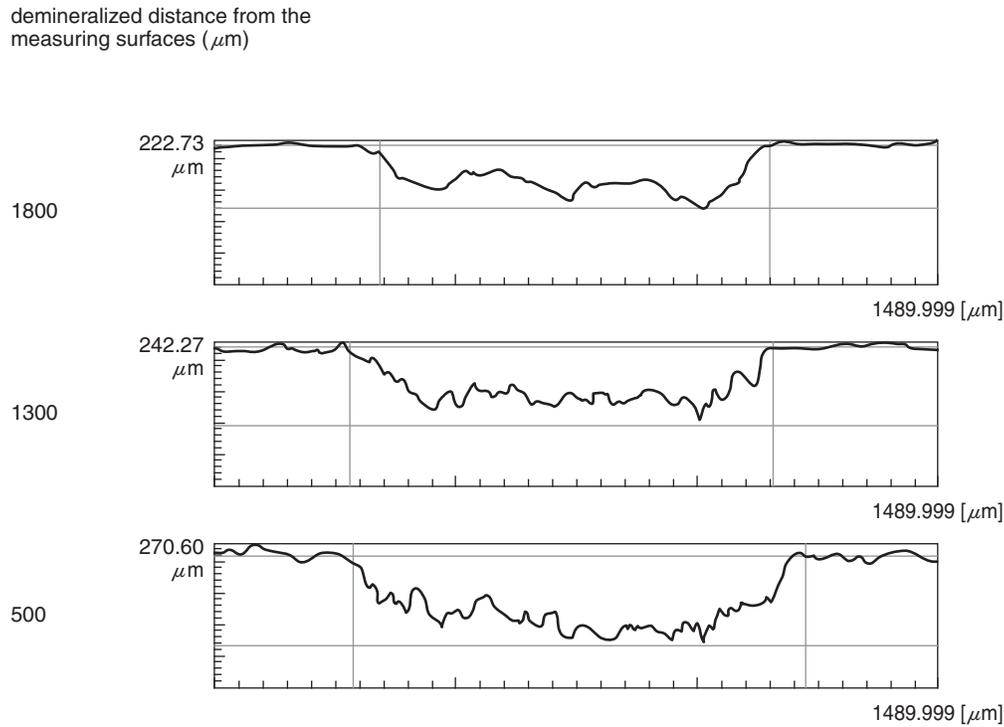1489.999 [μm]

270.60
μm

500

1489.999 [μm]

FIG. 4. Ablation width and ablation depth at 500, 1300, and 1800 μm from the measuring surfaces. In the DD group, ablation depth and width increased at 500, 1300, and 1800 μm from the measuring surfaces.

Dentin was considered to be sound when no significant differences in hardness were identified in comparison with the SD group at 1500–2000 μm. The intermediate demineralized layer was designated as lying between 1100 and 1400 μm deep. Hosoya et al. used dentin that was artificially softened at the cementoenamel junction, and ablated up to a hardness of 24.3 KHN, and they reported rehardening occurred following remineralization .[10] In this study, remineralization was expected to occur in the same area.

The Er:YAG laser has a wavelength of 2.94 μm and offers excellent water absorption efficacy. In this study, ablation volume was greater in the DD group than in the SD group, and this was solely attributable to differences in the absorption ability of the irradiated tissues, as the irradiation conditions were identical in both groups. Levels of in-

organic components have been reported to be reduced, and organic components such as collagen become exposed in demineralized dentin.[11–14] Gwinett reported that the volume of dentin collagen demineralized by acids depended on its moisture content, and that dentin collagen showed shrinkage under dry conditions.[13] Veis and Schlueter reported that collagen in demineralized dentin was different from, and more swollen than, skin collagen.[14] In other words, demineralized dentin represents dentin with no inorganic components, and its exposure to acid degenerated the collagen.[11] It has been suggested that such an exposed collagen network contains a large amount of moisture originating from saliva.[12] As this water absorbs laser energy, ablation volume increases due to the hardness of the demineralized dentin.
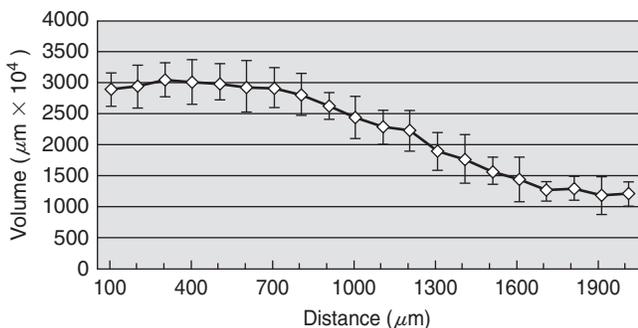
FIG. 5. Relationship between ablation volume and distance from the superficial surface in the DD group.
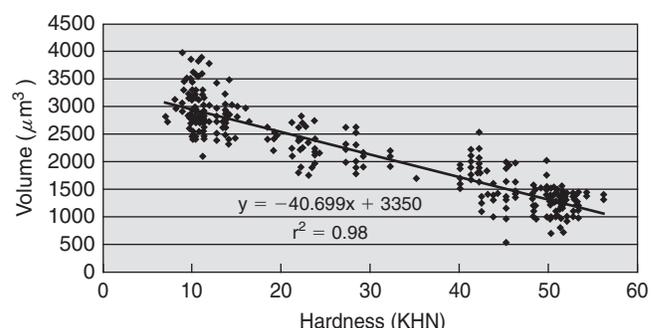
FIG. 6. Relationship between ablation volume and Knoop hardness at each measurement point.

$y = -40.699x + 3350$
$r^2 = 0.98$

In this study, the relationship between Knoop hardness and ablation volume was investigated, and an inverse correlation was confirmed between hardness and ablation volume. To avoid damaging sound dentin, an ablation method that selectively ablates only the first layer of carious dentin would be optimal. The present results show that the Er:YAG laser is useful for selective ablation of the top layer of carious dentin, because the ablation volume of demineralized dentin was greater than that of sound dentin.

This work represents the first in a series of experiments aimed at investigating new differential and selective ablation techniques for removal of demineralized dentin using the Er:YAG laser. The Er:YAG laser is capable of ablating carious dentin without damaging sound dentin.

Conclusions

A negative correlation was found between Knoop hardness and ablation volume using the Er:YAG laser ($y = -40.699x + 3350$, $r^2 = 0.98$). This demonstrated that the ablation volume of demineralized dentin was greater than that of sound dentin. The results suggest that the Er:YAG laser is capable of selectively ablating carious dentin without damaging sound dentin, via adjustment of output power and pulse rate.

Acknowledgments

Disclosure Statement

No competing financial interests exist.

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