Original Article

EFFECT OF Er:YAG LASER IRRADIATION ON ACID RESISTANCE TO BOVINE DENTIN IN VITRO

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Abstract

Resin bond strength to Er:YAG laser irradiated dentin has been reported to be lower than that of unlased dentin. The reasons have been much discussed, but not clarified. One hypothetical cause has been discussed that lased dentin is acid resistant, therefore, the etching effect of acid conditions decreases. The purpose of this study was to evaluate the acid resistance of laser-irradiated dentin and compare it with the dissolved mineral of Er:YAG laser irradiated dentin and unlased dentin. This experiment was a pilot study to assess the etching effect of pre-conditioner for resin bonding to lased dentin. Bovine dentin was irradiated by Er:YAG laser and immersed in 0.1M lactic buffer solution (pH 4.0). The dissolved Ca and P in the solution were then both measured. Dissolved Ca from lased dentin was not significantly different from that coming from unlased dentin (p>0.05). The molar ratio of Ca/P did not differ significantly between lased and unlased dentin, either (p>0.05). Under FE-SEM view before immersion, the dentin surface was covered with a smear layer in unlased dentin, but this layer was not clearly observed in lased dentin. These results suggested that the lased dentin had little or no resistance to lactic buffer solution.

Key words: Bovine dentin—Er:YAG laser—Acid resistance—Ca/P—SEM

INTRODUCTION

Since the early attempts to use ruby lasers in dentistry by Goldman et al., many experimental systems have used several other kinds of lasers, e.g. Nd:YAG and CO₂ lasers, for removal of hard tissues. The laser irradiation of the surface of the tooth substrate has also

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been reported to affect acid resistance\(^{19,20}\), so such systems have been clinically used for caries prevention\(^{23}\).

In 1988, Hibst and Keller\(^5\), and Keller et al.\(^{10}\) reported that the Er:YAG laser can remove dental hard tissue under water spray and that the irradiated hard tissues had minimal damage due to the high water absorption at the 2.94\(\mu\)m wavelength. Thus, reported pulpal effects of the Er:YAG laser in cavity preparation were supported to be equal to those in the cavities prepared by air-turbine\(^{16,21}\).

Prepared cavities irradiated by Er:YAG laser have usually been restored with plastic filling materials, especially with resin composites. The adhesion of dental hard tissue to resin has also been studied and dentinal adhesion requires the formation of a hybrid layer. A hybrid tissue of demineralized dentin and resin monomer that forms in the resin-dentin interface was first proposed by Nakabayashi in 1982\(^{14}\).

Kataumi et al.\(^8\) reported that the bond strength of resin composites to lased dentin was lower than that to unlased dentin and explained that the acid resistance of peritubular dentin induced by laser irradiation, resulting the etching effect of acid conditioner differed. Acid resistance of Er:YAG laser irradiated enamel was some reported\(^9\), but laser irradiated dentin was not as well studied. Micro cracks in dentin structure or denaturing of dentinal collagen have also been suggested as other reasons for the lower strength of resin bonding.

In the present study, we evaluated the effects of acid resistance to bovine dentin irradiated by Er:YAG laser used for demineralization by using an acid-etching biopsy with lactic buffer solution and comparing the results to those with unlased dentin.

**MATERIALS AND METHODS**

The procedure of this study is shown in Fig. 1.

**1. Laser apparatus**

The laser apparatus used in this study was a proto-typed Er:YAG laser (J. Morita Mfg. Corp., Kyoto, Japan). This apparatus consists of a power supply and a laser head. The wavelength was 2.94\(\mu\)m, the output energy settings ranged from 30 to 350 mJ/pulse at the control panel, the pulse repetition rates were 1, 3, 5, and 10 pulses per second (pps), and diameter of the contact probe was 600\(\mu\)m. In this study, the given output energy at the probe’s end was 100 mJ/pulse and 10 pps under water spray; these are the levels generally applied for caries removal in dental clinic. Energy levels were measured on demand with Lasermate-P (Coherent, CA, USA).

**2. Specimen preparation**

Seven extracted bovine teeth, frozen to maintain freshness, were defrosted and cut at the cervix and labiolingually. The exposed dentin of the cut surfaces was used in this study. One side of each surface was uniformly irradiated by Er:YAG laser (lased group), and the other side was not irradiated (control group). Dentin slabs of each specimen were prepared, and both sides of specimens were fixed to acrylic rods by inlay wax, which additionally covered the enamel and the unlased region of dentin to prepare the window.

**3. Demineralization**

Each specimen was immersed in 1ml of 0.1 M lactic buffer solution (pH 4.0) and kept
at 37°C for one hour. Then the specimens were removed from the solution, the solution was changed for new solution after specimen was rinsed with distilled water and then dried. The changing of the solution was again performed after 3, 6, 12 hours and 24 hours until the demineralization process was completed. After biopsy, a strip of tin foil was pressed on the specimen, and cut around the window five times for each specimen. The weight of the cut foils were measured with a microbalancer and used to calculate the window surface areas.

4. Quantitative analyses of Ca and P

The 1 ml aliquot of solution used for demineralization was separated into two 0.5 ml samples for analyzing calcium (Ca) and phosphorus (P) quantities. One of samples was diluted with 1M-HCl/0.5% La solution, and the amount of dissolved Ca was determined in fluid phase with an atomic absorption spectrometer (508A, Hitachi, Tokyo, Japan). The amount of dissolved P was measured by spectrophotometer (MPS-2000; Shimadzu Co, Kyoto, Japan) by the method of Chen et al. and the molar ratio of Ca/P was calculated.

5. Statistical analysis

There were seven samples in each group, and data were statistically analyzed with paired-sample t-test. The level of significance was set at p<0.05.

6. Field-emission scanning electron microscope (FE-SEM) observation

Six extracted bovine teeth were used, the specimens were prepared by the same method used for Ca and P analysis, demineralized in 0.1M lactic buffer solution (pH4.0) for 0, 1, 3, 6, 12, or 24 hours, and examined under FE-SEM (JSM-6340F; JEOL, Tokyo, Japan).

RESULTS

1. Amounts of dissolved Ca and the molar ratio of Ca/P

The quantitative analyses of Ca are shown in Table 1. The difference between the lased group and the control group was not significant regardless of immersion time, but the lased group tended to release less Ca then the control group.

The molar ratio of Ca/P is shown in Fig. 2. The differences between the groups were also not significant.

2. FE-SEM observations

FE-SEM views in each group before immersion and after immersion for 24 hours are
shown in Figs. 3 and 4. When the solution was not applied, the smear layer was observed and the dentinal tubular was not seen clearly in the control group. But in lased group, in contrast, the smear layer was not observed, the dentinal tubular was clearly observed, and the irradiated surface was imbricate-patterned. The smear layer was also absent in control on the specimen surface immersed for not less than one hour, and the imbricate pattern became less clear.

**DISCUSSION**

In the present study, we investigated the effect of irradiation by Er:YAG laser on the acid resistance of bovine dentin *in vitro*. We found no statistically significant differences between lased and unlased dentin.

The acid used in this study is 0.1M lactic buffer solution (pH 4.0), which is commonly used in the study of acid resistance. Some reports have used pH 4.0 to 4.5 of lactic or acetic buffer solution to investigate the acid resistance of lased enamel and reported it to be useful. Not only enamel but lased dentin was also reported to be significantly resistant to acid immersion; the surfaces of these dentins were reported to melt and caused it block of dentinal tubular. Acid resistance may cause the melting and changes in tooth
structure and may obstruct the penetration of solutions.

Some reports have suggested that the bond strength to lased dentin was lower than that of unlased dentin. As a pre-conditioning agent for resin-composite restoration, a gel-type or aqueous solution of phosphoric acid, citric acid, or self-etching primer is commonly used, and pHs are 0 to 3. Demineralization by a pH 4.0 solution is very slow. The fact that we found no differences in resistance to lactic buffer solution between the two groups suggests that the lower bond strength to lased dentin is not due to its acid resistance.

The molar ratios of dissolved Ca/P were also calculated in this study and were not significantly different between the two groups. These findings were similar to those in the report of Nakamura et al. and Kojy, who investigated the question by X-ray micro analyzer and calculated a ratio of 1.67, the theoretical rate of dentin structure. They suggested that their results indicated that the micro-structure of inorganic components are not changed by Er:YAG laser.

In this study, we did not obtain any significantly different results in the two groups, but the amount of dissolved Ca in lased group was slightly lower than in the control. In the FE-SEM view of not-immersed specimens, the dentin surface was covered with a smear layer and the dentinal tubular was not seen in the control. In contrast, the lased surface was not covered with a smear layer. Shimizu and Nakabayashi removed the smear layer from ground bovine dentin with an aqueous solution of EDTA and reported that the amount of Ca of the dissolved smeared layer in the solution was approximately 60 μg/cm². Therefore, the slight decrease in dissolved Ca in our lased group could have been due to the absence of the smear layer.

In the FE-SEM view, an imbricate-patterned surface was observed on the lased dentin. The Er:YAG laser has been reported to be able to remove hard tissue by micro-explosion or vaporization, caused by evaporation of hydroxyapatite when the laser affected the water in the tooth structure. These patterned surfaces and absence of the smear layer could have been due to differences in the abrasion mechanism.

In this study, the tin-foil method proposed by Brudevold et al. was used for the measurement of the window area. The method is useful for enamel surface investigation, and especially effective for measurement of window area because of the lased dentin surfaces that were uneven.

Our results suggested that the weakness of resin bonding to lased dentin was probably not due to the acid resistance. Further studies are needed to clarify the reason for the lower resin bonding to Er:YAG laser irradiated dentin.

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REFERENCES


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