Compositional and structural changes of human dentin following caries removal by Er,Cr:YSGG laser irradiation in primary teeth

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In this in vitro study, the compositional and structural changes of human dentin, and knoop harness of cavity floor following the removal of dental caries by Er,Cr:YSGG laser irradiation in primary teeth was compared with that of the conventional bur cavity. The results confirmed that laser irradiation revealed minimal thermal damage to the surrounding tissues, minimal thermal induced changes of dental hard tissue compositions, and favorable surface characteristic.
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INTRODUCTION

Many previous studies have reported that lasers are being considered as a potential replacement of conventional mechanical cutting and drilling system to remove diseased and healthy dental hard tissues. Since Goldman *et al*.[1-2] first demonstrated that caries removal was possible with the ruby laser in vitro, many researchers have investigated the effect of other lasers on decay prevention and carious dentin removal using the argon, CO^2 and Nd:YAG lasers.[3-5] However, these laser irradiations require relatively high-energy densities to vaporize the hard tissues, and they produce major thermal side-effects, such as melting, carbonization, the creation of fissures, cracks in the surrounding tissues, and an increase in the pulpal temperature.[6-9] Therefore, in order to find out a method to remove carious dental hard tissues or cavity preparations without any thermal side-effect, the potential applications of the erbium (Er:YAG and Er,Cr:YSG) lasers have been introduced and their application in the dental clinic have been expected. These lasers can ablate enamel and dentin more effectively due to their highly efficient absorption in both water and hydroxyapatite.[7] The ability of Er:YAG laser to remove enamel and dentin was found comparable to that achieved with the conventional dental drill,[10-11] and produces minimal thermal damage to the pulp or surrounding tissues, especially when irradiated with continuous water spray.[12-14] Animal histological studies also showed that pulp response to the Er:YAG laser appears to be similar to the response from high-speed hand piece application.[15] On the other hand, the Er,CR:YSGG laser, which uses a pulsed-beam system, fiber delivery, and a sapphire tip bathed in a mixture of air and water vapor, has been shown to be effective for soft-tissue surgery as well as for cutting enamel, dentin and bone.[16-19] When dental hard tissues were irradiated by the Er,Cr:YSGG laser accompanied with a water spray, not only could the temperature be suppressed, but cutting efficiency could be increased.[16-19] Histological studies confirmed that no pulpal inflammatory responses could be identified in Er,Cr:YSGG laser irradiated with a water spray.[20-21] Surface alterations of the enamel and dentin after Er,Cr:YSGG laser irradiation shows that these surfaces are associated with micro-irregularities and there was also the absence of a smear layer.[19] From the above results, it can be considered that Er,Cr:YSGG laser irradiation is favorable in the removal of carious dentin or cavity preparation in primary teeth, because it does not damage the dental pulp or surrounding tissues. However, before

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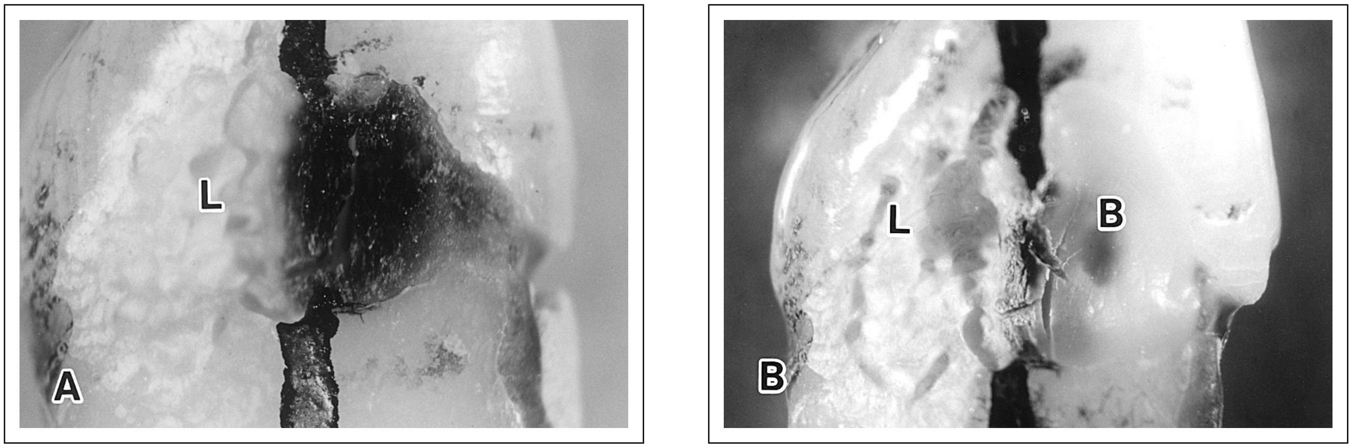

Figure 1. Experimental procedure used for this study. (A). Carious dentin with a deep brown to black color and medium consistency was selected (DIAGNOdent reading 91). One half of carious dentin removed with Er,Cr:YSGG laser irradiation (L) (original magnification x8). (B). Subsequently, the other half of each carious dentin lesion was removed with a mechanical bur treatment (B). In contrast to the relatively flat appearance of the bur treatment, the lased cavity surface (L) revealed an irregular pattern with absence of charring, carbonization or cracking of the dentin (original magnification x8).

application of this laser in the dental clinic, several other considerations need to be evaluated, especially there are still no reports on the compositional changes of human dentin following the removal of dental caries by Er,Cr:YSGG laser irradiation.

Therefore, this present study was performed to compare the compositional and structural changes of human dentin, and knoop hardness of the cavity floor following removal of dental caries by Er,Cr:YSGG laser irradiation with that of the conventional bur cavity, in primary teeth.

MATERIALS AND METHODS

Sample preparation

Twenty-five extracted human permanent teeth, with carious dentin on the proximal surfaces, were used. Each caries lesion was analyzed according to the color and hardness of the lesion. Carious lesions with a deep brown to black in color and medium consistency (it was resistance to probing and readily penetrated when tested with a sharp probe) were selected for this study. All lesions had no enamel coverage and it was easily accessible through the cavity openings. The extent of the carious lesions was assessed by means of KaVo DIAGNOdent 2095 (KaVo Dental GmbH, Jena, Germany), which provided a pulsed 655 nm laser beam directed into the tooth. When the incident light encountered a change in tooth substances, it stimulated fluorescent light of a different wavelength. This was translated, through the hand piece, into a number from 0 to 99. For the selection of carious dentin in this study, the following criteria were used: 0-20 = no caries; more than 20, the caries was deeper into the enamel or into the dentin. Carious lesions that scored more than 26 with this laser ray were used.

Experimental procedure

The experimental procedure used in this study for removing of carious dentin was that based upon a previous study.[14] Prior to the treatment, on the border of each lesion, two points were marked with a black marker pen along the centerline that longitudinally divides the lesion into two halves of approximately equal size. First, one half of the carious dentin were subjected to laser irradiation, subsequently the second half was treated by the mechanical method (Figures 1-A, B). It is unlikely that the laser irradiation could affect the adjacent carious dentin preserved for the mechanical treatment because previous studies have shown that the Er,Cr:YSGG laser can remove sound and carious dentin with minimal thermal effect on adjacent tissue.[16-19]

Removal of carious dentin was accomplished using the laser system, a Millennium™ Er,Cr:YSGG laser system (Biolase™ Technology Inc., San Clemente, CA) emitting a wavelength of 2.78 (µ with a repetition rate of 20 Hz and a beam spot size of 0.442 x mm^2 was used. The output power could be varied from 0 to 6 W. Each carious lesion was subjected to the Er,Cr:YSGG laser irradiation at 3 W (33.9 J/cm^2) output power (with 70% air level and 20% water level) with a non-contact method, until a total removal of the lesion.

Mechanical removal of carious dentin was done using round steel burs of appropriate size of 0.05 to 0.14, mounted on a micro-motor at 10,000 rpm. Gross carious dentin removal was verified according to the color and hardness of the lesion with a sharp explorer.

As carious dentin removal progressed and the treated cavity floor became deeper and closer to the underlying intact dentin layer, the treated cavity was carefully assessed by means of DIAGNOdent. The procedure was repeated until the DIAGNOdent shows a

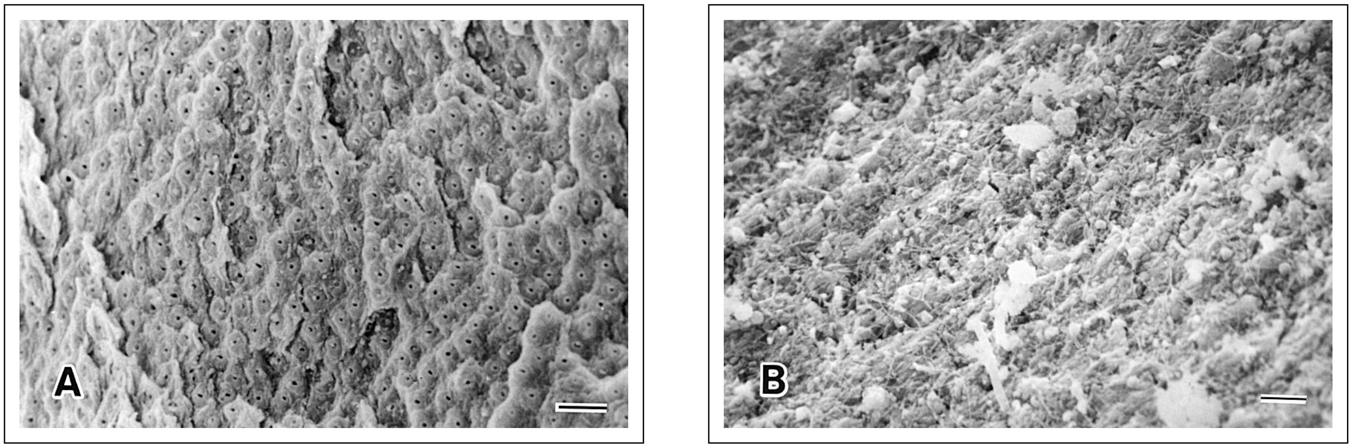

Figure 2. Representative scanning electron microscopic (SEM) photographs of dentin surface after cavity preparation with the laser (A) and bur treatment (B). Lased cavity was irregular and there was also absence of smear layer with the opening of the orifice of the dentinal tubules (original magnification x1000, bar represents 16 µm). Bur cavity surface on the other hand was covered with the debris-like smear layer (original magnification x2000, bar represents 10 µm).

value less than 20 and the following investigations were performed during and after the removal of carious dentin:

Measurement of temperature increase by thermography
Temperature change was measured at the time of each treatment using a thermovision device of 870 system (AGEMA, infrared systems AB, Danderyd, Sweden) linked to a personal computer (PC-AT) with a maximum resolution of 0.1° C around ambient temperature. Since the thermovision system generates a television-like image, thermal images were recorded on a computer in real time for later analysis. In order to analyze thermal images, a computerized thermal image processing system was applied (AGEMA, infrared systems AB, Danderyd, Sweden). The system captures and digitizes thermal image. The final results for each treatment were obtained as the mean and standard deviation (SD).

Evaluation of required time for carious dentin removal
The time required for caries removal was determined for each treatment, not including the working time for the inspection of the residual lesion. The differences in the times required for each treatment group were statistically analyzed by using Mann-Whitney U test, with a value of $P<0.01$ as being considered significant.

Atomic analysis and Knoop hardness measurement of the cavity floor
Twenty (20) cavities were cross-sectioned perpendicularly to the tooth axis through the middle of the treated cavity; one half was used for atomic analysis by SEM-EDX and the other half subjected to Knoop hardness test.

For atomic analysis by SEM-EDX, cut sections were fixed with a 10% formalin solution for 48 h, and then immediately perfused with a phosphate buffered solution of pH 7.3 at room temperature and rinsed with distilled water. The samples were dehydrated through a graded ethanol series (70, 80, 90, 95, and 100%) for 24 h at each concentration and then embedded in a polyester resin block (Rogolac, Nisshin, Tokyo, Japan). After the irradiated areas were flattened as much as possible by polishing, they were sputter-coated using a carbon-coating device (HUS-5GB, HITACHI, Tokyo, Japan and were examined by SEM-EDX (S-2500Cx, HITACHI, Tokyo, Japan and Model Delta V 1, Kevex, Foster city, CA) at 10 kV accelerating voltage; tilt angle at 350, and 3000 magnification.

For knoop hardness measurement of the cavity floor, cavity sections were embedded in epoxy resin, and the cross-sectional surfaces were polished. Since obtaining a Knoop hardness measurement of the cavity surface was impossible, recordings were obtained below the cavity floor; the hardness of the subsurface at the point 25 µm below the cavity floor was used as that of the cavity floor. The knoop hardness number (KHN) was measured in each treated cavity by the application of a 50-g load for 15 sec by means of a hardness tester. The mean of the measurements was used as the KHN of cavity floor dentin and statistical significant difference between the KHN of laser and bur groups was determined by Man Whitneys test.

Surface analysis of treated cavities
Cross sections of the remaining 5 cavities were examined by light microscopy and then followed by the SEM. Specimens were dehydrated in a grade series of aqueous ethanol (70%, 80%, 90%, 100% ethanol) for 24 hours in each solution. Dried by liquid CO_2 using a critical point dryer device (JCPD-3, JEOL, Tokyo, Japan), coated with platinum layer and observed by

scanning electron microscopy (SEM) (JSM-T220A, JEOL, Tokyo, Japan) at 20 kV.

RESULTS

Measurement of temperature increase by thermography

In the laser treated group, when the irradiation was performed with a water coolant, the temperature was found to have increase slowly and the mean temperature rise was 2.20 ± 1.30° C (Table 1). However, in the bur groups the mean temperature rise was 1.72 ± 1.24° C during the entire treatment time.

Table 1. Summary of the results found in this study (Mean ± S.D.)

	Laser cavity	Bur cavity
Thermal change (°C)	2.20 ± 1.30	1.72 ±1.24
Time required (sec)	27.50 ± 2.65[a]	13.10 ±1.29[a]
Knoop hardness (km/cm^2)	45.20 ± 4.56	43.50 ± 4.10
Ca (weight%)	35.45 ± 4.25[b]	27.40 ± 3.70[b]
P (weight%)	17.47 ± 3.57[c]	13.46 ± 3.28[c]
Ca/P	2.03	2.04

[a,b,c] Shows a significant difference (P<0.01)

Required time for carious dentin removal

The average required time of laser and mechanical treatments was 27.50 ± 2.65 sec and 13.10 ± 1.29 sec (mean ± SD), respectively (Table 1). The laser irradiation time was twice as long as the bur treatment and the differences were statistically significant (P<0.01). On the other hand, the working time required for the inspection of residual caries, which was not included in this measurement, was also longer, because evaluation of the completion of carious dentin removal was more difficult.

Atomic analysis and Knoop hardness measurement of the cavity floor

The results of atomic analysis showed that the quantities of Ca (Ca weight %) and P (P weight %) were increased significantly in the laser treated surface than that with the bur treatment. (Table. 1) (P<0.01). However, no significant differences were found between the Ca/P ratio of laser and bur cavities. Furthermore, there were no significant differences of the KHN of lased cavity floor with that of the bur cavity (Table 1).

Surface analysis of the prepared cavities

Light microscopic observation of cross sections of the laser treated surface revealed a rough or irregular surface with the absence of any charring, carbonization, or cracking of the dentin. SEM observation showed a scaly appearance or irregular surface due to micro-irregularities after laser irradiation (Figure 2-A). In addition, there was an absence of a smear layer; the orifice of dentinal tubules was exposed. The intertubular dentin had more ablation than the peritubular dentin, showing a protrusion of the tubules.

Light microscopic observations of the bur surface showed well-delimited cavity angles, floors and walls, clear margins and relatively smooth cavity floors. SEM observation revealed a relatively flat appearance and the surface was almost covered with a debris-like smear layer; dentinal tubule orifices were plugged (Figure 2-B).

DISCUSSION

The steps in caries removal or cavity preparation in primary teeth require precise operator control. Because, primary teeth have some particular characteristics; the pulpal outline follows the dentin-enamel junction more closely than in the case in permanent teeth, and the dentin is not as thick. Therefore, it follows that cavity preparation using a less traumatic caries removal system such as laser system may be favorable in pediatric dentistry.

When lasers have been applied to sound and carious dental hard tissues ablation, thermal side effects have been a major problem. However, a careful Er,Cr:YSGG laser technique using a non-contact mode and under continuous water spray did not produce any carbonization or cracking of the dentin in the present study. The results of the temperature increase also revealed that the surface mean temperature did not exceed 4° C (Table 1), which is believed to be that considered safe for pulp survival.[22] These findings correspond to some previous studies.[12,23] Furthermore, the results of SEM-EDX examination of the present study revealed that quantities of Ca or P in the lased areas were increased significantly compared with bur treatment. It is likely that during the laser irradiation, an increase of Ca or P could be achieved because organic components evaporated; these changes are thought due to temperature rise on the irradiated area.[24] However, no significant difference between Ca/P ratio of lased and bur cavities was found in the present study, which indicated that minimal thermal induced changes of dentin composition after the Er, Cr:YSGG irradiation could also be achieved. Animal histological findings of the previous studies have confirmed that no pulpal inflammatory responses could be identified in Er,Cr:YSGG laser irradiated with a water spray.[20-21]

Preliminary research has indicated that enamel and dentin etching with lasers have been shown to facilitate, or even improve over acid etching technique. The result of SEM observation of the present study revealed micro-irregularities and an absence of a smear layer; the orifice of dentinal tubules was exposed. Typically, these structures were similar to the surfaces after Er:YAG or Er,Cr:YSGG laser irradiation and have previously been described as scaly or flaky, or as an irregular surface.[10,11,19]

On the other hand, the dentin surface following the removal of dental caries with mechanical treatment showed a relatively flat appearance and exhibited a debris-like smear layer, which may interfere with adhesion, wetting, penetration, and hardness of the prepared cavity.[25-26] Although application of some acidic conditioners could remove smear layer, chemical changes may produce modification of the fraction of organic matter and decalcification of the inorganic component.[27-28] Laser technique might perform better because it leaves an etching behavior and does not damage the underlying tissues and dental pulp. The use of laser therapy also shows promise from the current research. Laser therapy facilitated or even improved bond strength.[29-32]

The results of required time for carious dentin removal has revealed that due to careful irradiation technique, cavity preparation time of approximately two to three times linger is needed with the laser system at 3 W output power as compared with a mechanical approach, which is likely to be inconvenient for some patients, particularly children. Longer preparation times during Er:YAG laser in permanent teeth was reported in a previous study.[14] In the clinic, it is possible to reduce the time by increasing the energy densities; in particular the out put power can be increased for cutting dentin, but it may increase the risk of thermal pulp damage and was therefore, avoided for this study.

The determination of complete caries removal seems to be an important factor during laser treatments. The color and hardness of the lesion have been used as two criteria for the detection of carious dentin. However, unlike mechanical treatment, the laser treatment did not provide sufficient tactile feedback to convey the precise hardness of the cavity floor and the operator could not feel precisely the differences in hardness between carious and intact dentin. In this study, a DIAGNOdent method was used as a clinical guide for detection of residual carious dentin. The usefulness of this device for the assessment of carious dentin removal has previously been confirmed.[33,5]

Microhardness measurement of the cavity floor confirms that Er,Cr:YSGG laser produce clean cut surface of the cavity and the possibility of residual softened dentin was minimum. No statistical significant differences were found in microhardness of laser and bur cavity floor; these results were correspondence to a previous study using the Er:YAG laser.[14] An increase of Knoop hardness of the cavity floor due to fusion of the dental hard tissues after CO_2 and Nd:YAG lasers irradiation was reported by the previous studies.[34-35] However, due to the temperature rise, higher energy densities of these laser irradiation actually decreased microhardness of dental hard tissues significantly and this condition is said not suitable for improving the properties of dental hard tissues.[36] Temperature rise during the laser irradiation also results in some major thermal side effects such as melting, cracking of enamel or dentin, and an increase in the pulpal temperature.[6-9] Such phenomenon was not noted in any of the laser cavities of the present study.

CONCLUSIONS

In the present study, atomic analysis indicated that the Ca/P ratio did not increase following laser irradiation, which suggests that Er,Cr:YSGG laser produce minimal thermal induced changes of dental hard tissue compositions. Knoop hardness of the lased cavity floor was also similar to the bur cavities. Stereoscopic and SEM findings suggested that laser device produce favorable surface characteristic and the step of acid etched technique could be easily avoided. It can be suggested that under adequate water spray or with a careful irradiation technique, cavities without thermal damage to the dental pulp can be produced; in addition, patients will experience less pain and anxiety with the laser device, so the dentist can offer this service to children as an alternative to needles and drills.

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