

# Morphological and Compositional Changes in Human Teeth following 9.6- $\mu\text{m}$ CO<sub>2</sub> Laser Irradiation In Vitro

Kaori Nagai<sup>a</sup>, Jun-Ichiro Kinoshita<sup>b</sup>, Yuichi Kimura<sup>c</sup>, Koukichi Matsumoto<sup>d</sup>

<sup>a</sup> Postgraduate Student, Department of Clinical Cariology and Endodontology, Showa University School of Dentistry, Tokyo, Japan.

<sup>b</sup> Assistant Professor, Department of Clinical Cariology and Endodontology, Showa University School of Dentistry, Tokyo, Japan.

<sup>c</sup> Associate Professor, Department of Clinical Cariology and Endodontology, Showa University School of Dentistry, Tokyo, Japan.

<sup>d</sup> Professor and Chair, Department of Clinical Cariology and Endodontology, Showa University School of Dentistry, Tokyo, Japan.

**Purpose:** The purpose of this study was to examine the morphological and compositional changes in human teeth following 9.6- $\mu\text{m}$  CO<sub>2</sub> laser use for cavity preparation.

**Materials and Methods:** Thirty-six extracted human teeth were randomly divided into two groups: a CO<sub>2</sub> laser-irradiated group and a turbine group. The 9.6- $\mu\text{m}$  CO<sub>2</sub> laser was used to irradiate 29 teeth for 20 s at 20 mJ pulse energy with 200 pps and 4 W output under water spray. Cavities in the turbine group were prepared using a diamond bur on 7 teeth. The morphology of samples was observed by stereomicroscopy, light microscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), or confocal laser scanning microscopy (CLSM). The calcium (Ca) and phosphorus (P) levels in each sample were measured by energy dispersive x-ray spectroscopy (SEM-EDX), and the results were analyzed statistically using the Mann-Whitney U-test.

**Results:** The cavity walls of the laser-irradiated group were not clear but whitish and relatively smooth when observed under light microscopy, and had rough boundary lines and wavy surfaces when viewed with SEM. There was no damage immediately underneath the irradiated surfaces when viewed with TEM. Mineral analyses (Ca, P) showed no statistically significant difference in the composition of the enamel or dentin after using a bur, but significant differences in weight percentage of the P level were found after laser irradiation.

**Conclusion:** It was demonstrated that 9.6- $\mu\text{m}$  CO<sub>2</sub> laser shows potential for clinical application in cavity preparation without harming surrounding dental hard tissues.

**Keywords:** 9.6- $\mu\text{m}$  CO<sub>2</sub> laser, human teeth, morphological change, compositional change, confocal laser scanning microscopy (CLSM).

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After the ruby laser was developed in 1960, the application of lasers in dentistry has been investigated since 1964. The use of various lasers for the ablation of caries and cavity preparation has also been examined. It was reported that exposure of intact dental tissues to a ruby laser beam resulted in a glass-like fused appearance of enamel with burned dentin and an

increase in enamel acid resistance under specific parameters.<sup>1,2</sup> When human vital teeth were irradiated with ruby laser, the volunteer experienced no pain, but a burning odor was evident.<sup>3</sup> Studies using ruby and Nd:YAG lasers have been reported, but the results showed drawbacks, such as dental pulp damage, dentin carbonization, and enamel cracking.<sup>4,5</sup>

To ablate dental hard tissues without carbonization, the development of a new type of laser was required. The Er:YAG laser was found to be able to remove both enamel and dentin.<sup>6,7</sup> No thermal damage was observed in surrounding tissues when cavity preparation was performed with Er:YAG laser on extracted human teeth under water cooling, but molten lava-like surfaces and irregular structures were observed when the laser beam was applied without water mist.<sup>8,9</sup>

As hydroxyapatite more readily absorbs the Er:YSGG laser beam of 2.78  $\mu\text{m}$  wavelength than the Er:YAG laser,<sup>10</sup> it was thought that the Er,Cr:YSGG laser beam of 2.79  $\mu\text{m}$  wavelength would be better absorbed by calcified dental tissues than would the Er:YAG laser. In an animal study which observed the canine dental pulp responses when using Er,Cr:YSGG laser, the pulp appeared to be more or less fibroblastic and tertiary dentin formation was induced.<sup>11</sup> The hydrokinetic system of the Er,Cr:YSGG laser was generally considered to be an ideal laser system for cutting dental hard tissues without damage to either surrounding tissues or the pulp inside.

On the other hand, CO<sub>2</sub> laser was considered to be useful for removing soft tissues,<sup>12,13</sup> and in osteotomy, the transversely excited atmospheric pressure (TEA) CO<sub>2</sub> laser is described as an instrument to ablate mineralized tissue with minimal side effects. It has been reported that after TEA CO<sub>2</sub> laser irradiation, the cavity edges were sharp, there was no thermal damage such as carbonization or necrosis on the cavity walls, and the depths of cavities were obviously near the bone marrow when irradiation time was lengthened, but thermal damage was limited.<sup>14</sup> Rapid superpulsed (SP) CO<sub>2</sub> laser irradiation avoids thermal accumulation and causes less damage to the tissues than continuous wave (CW) CO<sub>2</sub> laser. Both SP and CW CO<sub>2</sub> laser irradiation delayed the healing of bone fractures in contrast to the bur technique, but CW laser-treated fractures required around 4 more weeks to heal than did those treated with SP laser.<sup>15</sup> It was found that the short-pulsed CO<sub>2</sub> laser of 9.6- $\mu\text{m}$  wavelength yielded the same healing time in bone as Er:YAG laser when used for osteotomy.<sup>16</sup> It was thought that the CO<sub>2</sub> laser beam would be well absorbed by enamel because of its wavelength of 10.6  $\mu\text{m}$ ,<sup>2</sup> which was theoretically recommended for application to enamel and dentin surfaces. In 1972, the chemical and physical changes of enamel and dentin<sup>17-20</sup> and the acid resistance of enamel<sup>21</sup> induced by CO<sub>2</sub> laser irradiation were reported. It was confirmed that CO<sub>2</sub> laser provided acid resistance to enamel more effectively than ruby laser when extracted human teeth were irradiated with a

pulsed CO<sub>2</sub> laser,<sup>21</sup> but studies using continuous wave (CW) CO<sub>2</sub> laser demonstrated the occurrence of cracking on dentin at certain parameters.<sup>22</sup> Because CO<sub>2</sub> laser irradiation resulted in fusion of hydroxyapatite,<sup>17</sup> it was applied to dental hard tissues for filling pits or fissures, caries prevention, enamel etching, and laser pretreatment of dentin.<sup>23-26</sup> The dental pulp temperature did not exceed 6°C when irradiated with TEA CO<sub>2</sub> laser beam.<sup>27</sup> The walls of cavities were sharp and without carbonization or cracks, and the surfaces were comparable to those prepared by turbine.<sup>28</sup> This result agreed with a previous study,<sup>29</sup> which also reported no cracking or carbonization.

When the wavelength changed from 10.6  $\mu\text{m}$  to 9.6  $\mu\text{m}$ , CO<sub>2</sub> laser irradiation ablated dental hard tissues more effectively than irradiation at 10.6  $\mu\text{m}$  wavelength, although the ablation mechanism appeared to be the same.<sup>30</sup> Cavities were clinically prepared on human third molars using 9.6- $\mu\text{m}$  CO<sub>2</sub> laser and the dental pulps were undamaged.<sup>31</sup>

Although the cavity conditions and response of dental pulps after 9.6- $\mu\text{m}$  CO<sub>2</sub> laser irradiation have been investigated, few studies to date have reported the morphological and compositional changes after irradiation. The objective of this study was to investigate morphological and compositional changes of cavity walls after 9.6- $\mu\text{m}$  CO<sub>2</sub> laser was used for cavity preparation on extracted human teeth.

## MATERIALS AND METHODS

### Sample Preparation

After informed consent in accordance with the Declaration of Helsinki was obtained from the patients, 36 extracted human teeth (14 incisors, 12 premolars, 10 molars) without evident carious lesions on the surfaces were used in this study. All teeth, which were extracted due to periodontal or orthodontic reasons, were permanent teeth, and had been stored in 10% formalin to prevent drying. Cementum was gently removed from the roots with a hand scaler and dentin was exposed. The teeth were randomly divided into 2 groups: a CO<sub>2</sub> laser group (29 teeth) and a turbine group (7 teeth). In the laser group, the centers of the labial/buccal and lingual/palatal surfaces of tooth crowns were chosen for enamel, and the centers of labial/buccal and lingual/palatal cervical zones on roots 7 mm below the cemento-enamel junction (CEJ) were chosen for dentin. Both the labial/buccal and lingual/palatal sides were irradiated. Cavities in the turbine

**Table 1 Experimental design**

	Laser group		Turbine group	
	29 teeth		7 teeth	
Total	58 enamel sections	58 dentin sections	14 enamel sections	14 dentin sections
Light microscope (H-E dye)	4	4	2	2
SEM	36	24	8	4
TEM	0	12	0	4
CLSM	10	10	0	0
SEM-EDX	8	8	4	4

group samples were prepared using a diamond bur (#103, Shofu; Kyoto, Japan) under a water spray of 50 ml/min. They were made at the same sites as the samples of laser group. All teeth were bisected longitudinally into 2 parts along the mesiodistal axis, and each half was further cut into 2 parts at the CEJ. There were 58 enamel and 58 dentin samples in the laser group, and 14 enamel and 14 dentin samples in the turbine group (Table 1).

### Laser Irradiation

In this study, a prototype 9.6- $\mu\text{m}$  wavelength CO<sub>2</sub> laser device (Yoshida; Tokyo, Japan) was employed. The focal distance was 70 mm, the spot diameter 1.5 mm, output power 4 W, repetition rate 200 pps emitting in 90  $\mu\text{s}$ -long pulses, total pulse energy 20 mJ/pulse, and energy density 2038 J/cm<sup>2</sup>. These parameters were chosen for this study as they had been shown to be the most efficient in cutting hard dental tissues in our preliminary studies, which confirmed the correctness of the recommended parameters by the manufacturer (Yoshida) of this experimental device. The laser device had a 0.5-mm diameter focal point, automatically irradiated in spirals, which resulted in a larger irradiated area measuring 1.5 mm in diameter (scanning speed related to the laser device was 0.793 s).

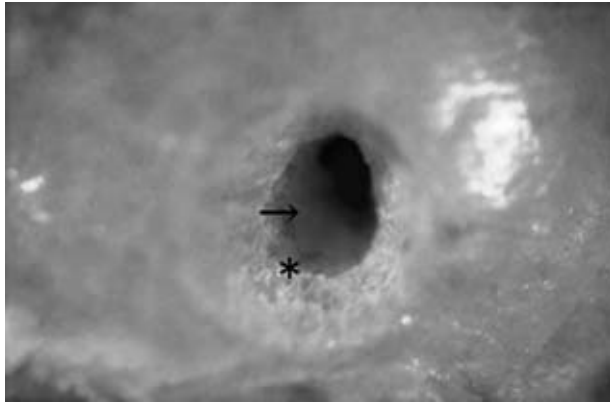
Samples of the laser group were irradiated for 20 s under a water spray of 10 ml/min. The distance to the focal point was determined by using a straight metal wire of 70 mm which was attached to the tip of laser handpiece. The handpiece was fixed at right angles to

the surface by a single operator who was well experienced and blinded to the nature or purpose of this study.

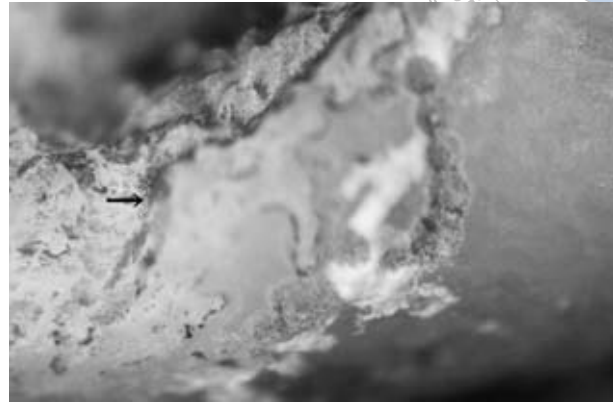
### Microscopic Observation

After all samples were observed under a stereomicroscope (SMZ-10, Nikon; Tokyo, Japan), the samples of the laser group were randomly divided into 5 subgroups and those of turbine group were divided into 4 subgroups: a light microscopic subgroup (enamel-laser 4, dentin-laser 4, enamel-turbine 2, dentin-turbine 2), a scanning electron microscopic (SEM) subgroup (enamel-laser 36, dentin-laser 24, enamel-turbine 8, dentin-turbine 4), a transmission electron microscopic (TEM) subgroup (dentin-laser 12, dentin-turbine 4), a confocal laser scanning microscopic (CLSM) subgroup (enamel-laser 10, dentin-laser 10), and an energy dispersive x-ray spectroscopic (SEM-EDX) subgroup (enamel-laser 8, dentin-laser 8, enamel-turbine 4, dentin-turbine 4) (Table 1).

The samples in the light microscopic subgroup were dehydrated by immersion in serial dilutions of aqueous ethanol (70, 80, 90, 95, and 100%) and embedded in Shiojirin (Shiojirin-E10, Showa Yakuhin Kako; Tokyo, Japan). Each Shiojirin block was sectioned to a thickness of 20  $\mu\text{m}$ , and each slice was stained with hematoxylin-eosin (H-E) solution and observed by light microscopy. For the SEM subgroup, all specimens were dehydrated by immersion in serial dilutions of aqueous ethanol (70, 80, 90, 95, and 100%). After drying with liquid CO<sub>2</sub> using a critical-point dryer (JCPD-3, JEOL;



**Fig 1** Representative stereomicroscopic photograph of laser-irradiated enamel at a magnification of 10X. The edge of the laser-irradiated area was a shallow slope and became a whitish nebula. The dentin wall appeared almost straight with a clear boundary to enamel cavity. Almost no carbonization was observed after irradiation in enamel. Deeper inner wall could be observed (arrow). The boundary of the shallow slope (asterisk) was not clearly observed, and the whitish margins were not smooth but uneven.



**Fig 2** Representative stereomicroscopic photograph of laser-irradiated dentin at a magnification of 40X. The wall of cavity was uneven and whitish compared with the nonirradiated area, and the original luster was lost. A part of the wall was carbonized. Signs of spiraling irradiation (arrow) were recognized on dentin, although this was an artifact created when the operator failed to keep the handpiece stationary.

Tokyo, Japan), samples were sputter coated with platinum, and observed under field-emission SEM (FE-SEM, E-1030, Hitachi; Tokyo, Japan) using an accelerating voltage of 15.0 kV. For the TEM subgroup, all samples were demineralized by EDTA, dehydrated by immersion in serial dilutions of aqueous ethanol (70, 80, 90, 95, and 100%) and embedded in epoxy resin (TAAB EPON 812, TAAB Laboratories Equipment; Tokyo, Japan). Approximately 1- to 2- $\mu$ m-thick sections were prepared and observed by TEM (H-7000, Hitachi). Samples in the CLSM subgroup were dehydrated by immersion in serial dilutions of aqueous ethanol (70, 80, 90, 95, and 100%), stained with rhodamine B (0.479 mg/10 ml PBS) and observed by CLSM (LSM510META, Carl Zeiss; Jena, Germany) argon laser at 458 nm, 50- $\mu$ m intervals for enamel and 10- $\mu$ m intervals for dentin.

Some areas on the unlased enamel and dentin around the cavity in each sample were randomly chosen as control, and the calcium (Ca) and phosphorus (P) level in enamel and dentin of control and cavities of both groups were measured using SEM-EDX (Quantum  $\delta$ -IV type: KeveX; Foster City, CA, USA) after they were sputter coated with carbon. The Ca and P levels were analyzed statistically using the Mann-Whitney U-test for comparison between control and turbine groups, control and laser groups, and turbine and laser groups with a level of significance of  $p < 0.001$ .

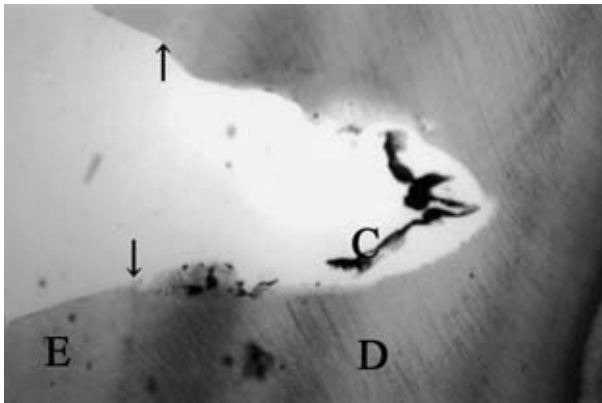
## RESULTS

### Stereomicroscopic Findings

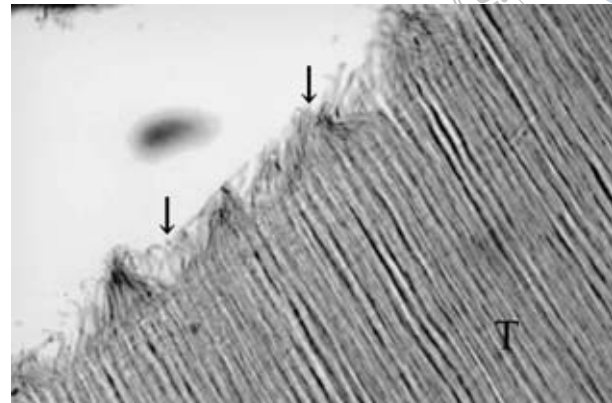
The stereomicroscopic observation of the laser group samples showed deep round holes on tooth surfaces. The boundaries of turbine group samples were clear, whereas those of laser group samples were indistinct and whitish, and the luster was lost (Fig 1). The shallow circumference wall, which corresponded to the enamel layer, had a slope with a different angle from the deeper wall, which resembled a right-angle slope. Signs of carbonization were observed on enamel. The dentin floors of the cavities were carbonized. The inner walls which seemingly corresponded to dentin were rough when examined at a high magnification of 40X (Fig 2). Slightly dark-colored carbonization was detected in some areas of the cavity walls, and the other areas appeared white. The laser-irradiated areas were lusterless, and the margins were unclear and rough.

### Light Microscopic Findings

The mean depth of the cavities after laser irradiation was 3 mm (Fig 3). The floor of the cavity extended into dentin and was wedge shaped. The boundaries between enamel and dentin were clearly visible, and the



**Fig 3** Representative light microscopic photograph of a sample from CO<sub>2</sub> laser group at a magnification of 200X. The cavity margin in enamel (E) was clear and the angle was changed at EDJ (arrows). The dentin (D) wall was uneven and a carbonized layer (C) near the bottom was observed.



**Fig 4** Representative light microscopic photograph of a sample from CO<sub>2</sub> laser group at a magnification of 600X. Under this magnification, the dentinal tubules (T) were exposed and looked fluffy (arrows), due to removal of the carbonized layer.

angles of the cavity walls were changed at the boundaries, which corresponded to the stereomicroscopic observations. The walls of the laser group samples were relatively smooth, and a part of the surface near the bottom exhibited a carbonized layer approximately 1  $\mu\text{m}$  thick. At a magnification of 600X, the carbonized layer appeared separated from the dentin surface which looked fluffy, and the orifices of dentinal tubules were open, but no changes were observed right beneath the surface (Fig 4).

The samples of the turbine group showed flat box-shaped cavities after drilling with the air turbine. No carbonization was recognized on any walls, but there were thin smear layers on the surfaces of the cavities.

### SEM Findings

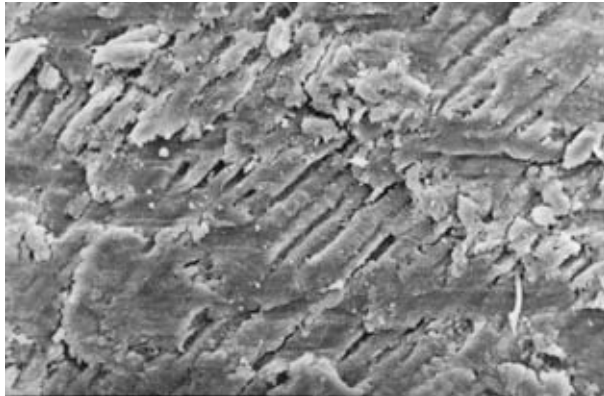
The enamel surfaces of turbine group samples showed many small parallel scratch marks caused by the diamond particles on turbine bur (Fig 5). Enamel columns exposed by friction were observed, and much smear layer remained on the flat excavated surfaces. Dentin-turbine group samples also had a thick smear layer on the walls, and the openings of dentinal tubules were not visible.

The margins of the cavity in the enamel laser group samples were not smooth and appeared wavy due to

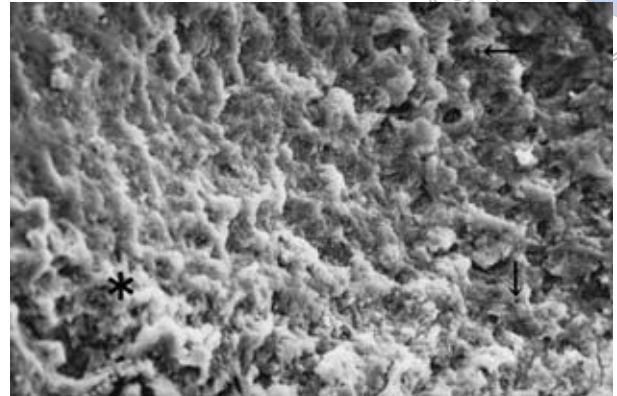
laser focus movement. The centers of irradiated areas were very rough and the side areas looked like lava, but the signs of recrystallization were more prominent on the side walls of the shallow dish cavities than on those at the center. The enamel surfaces had melted and showed a lava-like appearance. The pits seen on the lava-like surface were not original tooth structure, but recrystallization after melting (Fig 6).

Cavities in dentin-laser group samples showed appearances similar to those of the enamel-laser group samples such as a rough, wavy margin. Low magnification observation (200X) showed one deep crater and a few shallow ones (Fig 7). At the very center of the deepest crater, some small projections were observed, which were considered to be molten dentin mass. The wavy appearance was similar to that observed in enamel-laser samples. The walls of bowl-like cavities were smoother than those of enamel. At higher magnification (1000X), the lava had a honeycomb appearance, which was slightly different from enamel-laser samples (Fig 8). Recrystallized dentin was radially spread with the center at the bottom, and the pits observed among the melting mass were considered openings of dentinal tubules, but they were a third less in area than intact dentin walls.

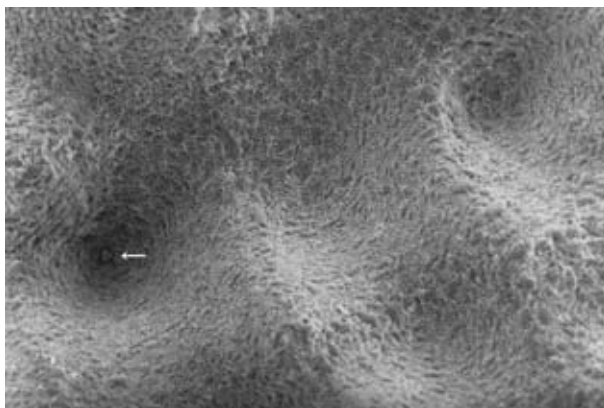




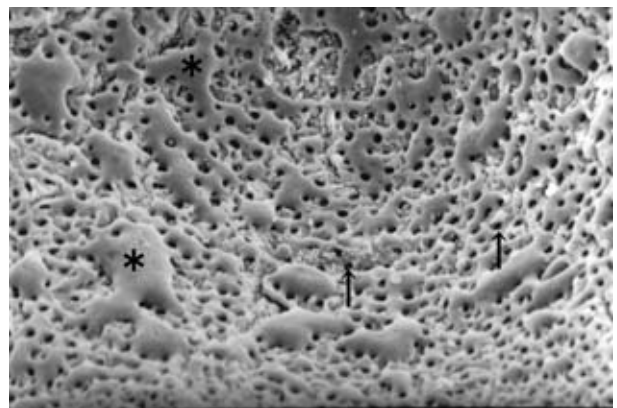
**Fig 5** Representative SEM photograph of a sample from the enamel-turbine group at a magnification of 1000X. Enamel columns were exposed after cavity preparation and much debris was visible.



**Fig 6** Representative SEM photograph of a sample from the enamel-laser group at a magnification of 1000X. The melted and recrystallized surface resembled lava (asterisk). Pits (arrows) evident on "lava" were not original tooth structure, but the recrystallization after melting.



**Fig 7** Representative SEM photograph of a sample from the dentin-laser group at a magnification of 200X. There were a few craters around the deepest one. The surface was smoother than that of enamel. Arrow shows the intended focus point of this cavity at the bottom.

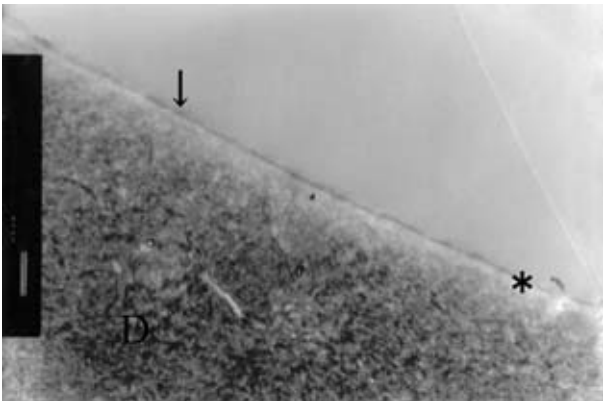


**Fig 8** Representative SEM photograph of a sample from the dentin-laser group at a magnification of 1000X. Honeycomb lava structure was observed, and a part of dentinal tubules was closed but most of dentinal tubules were open (arrows). Recrystallized dentin (asterisk) was radially spread on the cavity surface with the focus of the laser beam at the center.

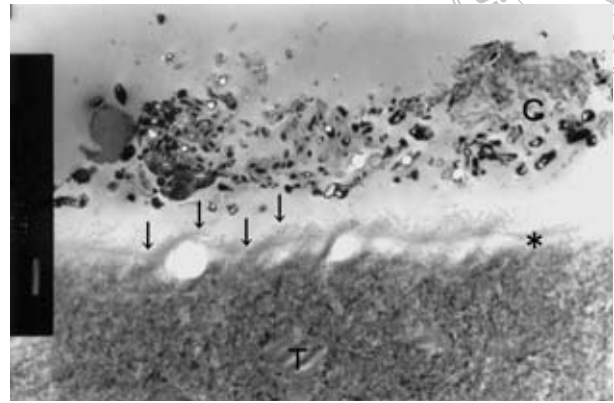
## TEM Findings

The surfaces of dentin-turbine group samples were smooth and flat, but some dark thin layers which corresponded to smear layers were evident on surfaces of the dentin walls (Fig 9). The thickness of those layers was less than 1  $\mu\text{m}$ , and the tissues beneath them also looked sound.

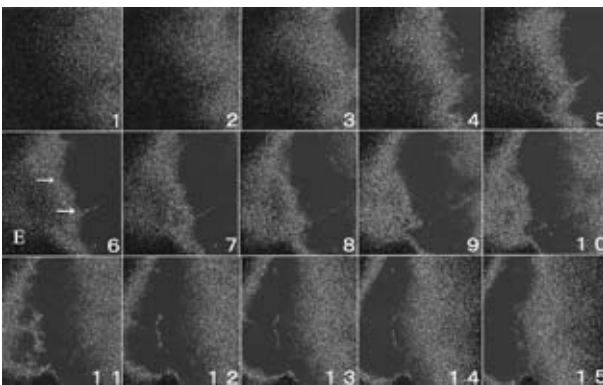
The dentin surfaces of laser group samples were rough and uneven (Fig 10). The carbonized mass looked like a galaxy consisting of black balls, and appeared separated from the dentin body, which had an approximately 3- $\mu\text{m}$ -thick nonstructural transparent layer which corresponded to the fluffy zone observed by light microscopy in samples of the same group (Fig 4). Many collagen fibers were detected underneath the transparent layer. There were many clusters which



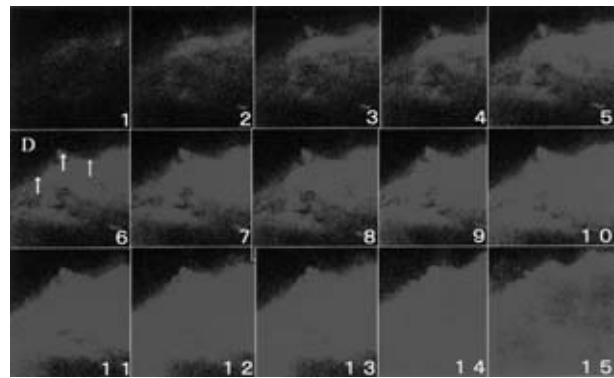
**Fig 9** Representative TEM photograph of a sample from the dentin-turbine group at a magnification of 3000X. The surface was smooth (arrow), but denatured by frictional heat. Underneath the denatured layer (asterisk), there was no sign of damage in dentin structure (D).



**Fig 10** Representative TEM photograph of a sample from the dentin-laser group at a magnification of 2500X. The dentin surfaces were rough and uneven (arrows). The carbonized mass (C) was separated from the dentin. No structural white layer (asterisk) was recognized on the surface. Underneath the surface, many collagen fibers and dentinal tubules (T) were recognized. The tissues beneath the white layer were probably not damaged by laser irradiation.



**Fig 11** Representative CLSM photographs of a sample from the enamel-laser group at a magnification of 200X. The cavity margins (arrows) of enamel (E) were irregular and uneven, and the form was complex.



**Fig 12** Representative CLSM photographs of a sample from the dentin-laser group at a magnification of 200X. The cavity margins (arrows) of dentin (D) were irregular and uneven. No dentinal tubule was recognized, but a complex cavity with many undercuts was identified.

were 0.3 to 1.2  $\mu\text{m}$  in size near the surfaces, but the tissues beneath this layer were intact.

### CLSM Findings

The three-dimensional (3-D) structures of lased cavities were observed by CLSM (Figs 11 and 12). The cavity margins of enamel and dentin were irregular and un-

even, and the shapes were very complicated with some undercuts. The morphology coincided with the findings by stereomicroscopy, light microscopy, and SEM, which showed the existence of a recrystallized mass and many shallow craters on the cavity walls. The particular micromorphological changes were not observed, except for the complexity of shape. The dentinal tubules were not visible in dentin-lased samples.

**Table 2 Concentrations of Ca, P and Ca:P ratio in enamel after laser or bur**

	Ca-W%	P-W%	Ca:P WR	Ca:P AR
Untreated enamel	36.512 ± 0.511	17.816 ± 0.565	2.051 ± 0.053	1.585 ± 0.041
Laser	38.854 ± 0.813	18.703 ± 0.819	2.080 ± 0.061	1.606 ± 0.048
Turbine	37.920 ± 0.740	18.090 ± 0.390	2.097 ± 0.029	1.621 ± 0.023

Ca:P WR = Ca:P weight ratio; Ca:P AR = Ca:P atomic (mol) ratio.  
No significant difference from untreated enamel ( $p < 0.001$ ).

**Table 3 Concentrations of Ca, P and Ca:P ratio in dentin after laser or bur**

	Ca-W%	P-W%	Ca:P WR	Ca:P AR
Untreated dentin	27.440 ± 0.560	13.210 ± 0.290	2.078 ± 0.059	1.606 ± 0.045
Laser	* 37.383 ± 2.220	* 18.030 ± 1.083	2.074 ± 0.060	1.604 ± 0.044
Turbine	28.530 ± 0.540	13.970 ± 0.360	2.044 ± 0.035	1.579 ± 0.028

Ca:P WR = Ca:P weight ratio; Ca:P AR = Ca:P atomic (mol) ratio.  
Asterisks show significant differences from untreated dentin ( $p < 0.001$ ).

## SEM-EDX

Changes in mineral content were measured by SEM-EDX, and the mean concentrations of Ca, P and Ca:P ratio in enamel and dentin following turbine or laser irradiation are shown in Tables 2 and 3. Percentages of Ca and P slightly increased in enamel both after turbine drilling and after CO<sub>2</sub> laser irradiation, but there was no statistically significant difference in the percentages of Ca, P and Ca:P ratio in enamel (Table 2).

After laser irradiation, Ca and P weight percentages increased significantly, while there was no change after drilling. The Ca:P ratio did not change in the samples of turbine and laser groups. Only the dentin-laser group was significantly different from the original dentin surfaces in Ca weight and P weight percentages ( $p < 0.001$ ).

## DISCUSSION

### Laser Device and Irradiation Conditions

Optically speaking, the human tooth has been considered a material structure that can transmit, scatter, and absorb light.<sup>32</sup> The absorption and transmission of a

laser beam applied to human teeth depends much on the wavelength of the laser beam.<sup>5</sup> At the usual wavelength of 10.6  $\mu\text{m}$ , CO<sub>2</sub> laser energy was mainly absorbed by dental enamel.<sup>2</sup> At the wavelength of 9.6  $\mu\text{m}$ , it was strongly absorbed not only by water, but also by other organic components of both enamel and dentin such as collagen.<sup>30,31</sup> In enamel, CO<sub>2</sub> laser wavelengths of 9.32  $\mu\text{m}$  and 9.9  $\mu\text{m}$  are mostly absorbed by hydroxyapatite.<sup>33</sup> Therefore, it was thought that a wavelength of 9.6  $\mu\text{m}$  would modify hard tissues efficiently by vaporization and cause less damage.

It has been reported that there is no relationship between the selected laser parameters and the observed surface changes in the range of 75 to 100 J/cm<sup>2</sup>.<sup>34</sup> Moshonov et al suggested that the 9.6- $\mu\text{m}$  CO<sub>2</sub> laser energy levels, 7 W on enamel and 3.5 W on dentin, for cavity preparation were the ideal settings for excavating enamel and dentin.<sup>35</sup> The parameters (output 4 W, 200 pps, 90- $\mu\text{s}$  pulses, pulse energy 20 mJ/pulse, and energy density approximately 10 J/cm<sup>2</sup>) enabled cutting of hard tissues including enamel, but dentin was carbonized because of inadequate water mist or a higher than recommended power output level. Consequently, it was suggested that the laser power output levels, 4 W on enamel and much less than 4 W on dentin, were sufficient for effective cavity preparation.



### Analysis of Stereomicroscopic Findings

CO<sub>2</sub> laser energy is well absorbed by enamel and dentin surfaces; these tissues do not transmit the laser beam.<sup>36</sup> Clear-cut pinholes can be made and the depth increases according to the amount of energy.<sup>10</sup> The CO<sub>2</sub> laser at wavelengths of 9.3 μm, 9.6 μm, 10.3 μm, and 10.6 μm were compared on enamel, and it was found that roughness and air bubbles appeared at the center of the laser-irradiated area at the wavelengths of 9.3 μm and 9.6 μm, while they were not observed with the wavelengths of 10.3 μm or 10.6 μm.<sup>37</sup>

In the present study, whitish areas caused by melting after laser irradiation were found on enamel, and signs of carbonization were observed on dentin in spite of using water cooling.

The reason for this difference to previous studies was that the laser beam was absorbed too completely by collagen and water at the wavelength of 9.6 μm. Because the highly organic dentin absorbed this laser energy more than enamel did, cooling the dental hard tissue by water spray was not sufficient or the laser energy was too high.

### Analysis of Light Microscopic Findings

It has been demonstrated that a lower output (0.5 W, 100 μs, 0.1 s) had no effect on enamel, but a deep cavity reaching well into dentin was formed by higher output (9.8 W, 200 μs, 0.1 s).<sup>38</sup> A moderate output of 4 W was used in the present study, because the dentin in the above-mentioned report appeared too severely damaged. As a result, the dental hard tissues were not ablated as well as in the study above, and uneven cavities were formed. It was suggested that the carbonized layer, which consisted of denatured dentin, was quite susceptible to mechanical stimulation, making it easy to eliminate with water pressure and laser-induced microexplosions. Water spray could not reach the bottom of the wedge shaped cavity, leaving the carbonized layer at the floor of the cavity. In the area where the carbonized layer was removed, the dentinal tubules were open and appeared fluffy or fuzzy. There was no sign of recrystallization under the removed carbonized area. It is not clear whether this fluffy layer exerts an influence on adhesive resin or other restorative materials.

### Analysis of SEM Findings

In this study, neither enamel nor dentin surfaces showed debris or smear layer after laser irradiation, which was contrary to the samples of turbine group. The pressure of the water spray from the handpiece and microexplosion at focus points may have removed the weakly attached substances.

On enamel and dentin irradiated by this laser, continuous shallow concavities were detected. This could be because the laser beam, which originally had a 0.5-mm-diameter focal point, automatically irradiated in spirals, which resulted in a larger irradiated area measuring 1.5 mm in diameter (scanning speed related to the laser device was 0.793 s). Under high magnification, the pits on irradiated enamel were thought to be air bubbles after boiling of the enamel crystal substance, while the pits on irradiated dentin were recognized as open dentinal tubules, though many dentinal tubules were closed by lava-like melted dentin. At the center of the cavity floor in dentin, there was a focused denatured area which received the maximum energy from irradiation. In contrast, many shallow bowl-like concavities on the dentin cavity wall showed no difference in depth. This indicated that the many waves on the lased surfaces were not due to the unconscious movement of the handpiece by the operator, but were rather a peculiar aspect of this type of laser. It was thought that the spiral irradiation increased the absorption rate with less reflection, and the 9.6 μm wavelength enabled more efficient absorption of the beam by hydroxyapatite. The CO<sub>2</sub> laser beam may be 90% absorbed within a distance of only 100 μm or so, and a 20 W beam focused into a 2-mm-diameter spot would penetrate nearly 2.5 mm of tissue in one second,<sup>36</sup> but there was not enough information to confirm this at this stage of study.

Many dentinal tubules were closed and their surface was modified after irradiation. Based on this observation, it can be postulated that some increase in mechanical strength for resin adhesion and prevention of hypersensitivity after laser irradiation could be expected, because the closed dentinal tubules would shut out thermal stimulation, although another bond strength experiment is necessary to confirm this.

### Analysis of TEM Findings

TEM photographs in this study using CO<sub>2</sub> laser showed similarities to those of Nd:YAG laser, which means the outer layer revealed the dissolved area together with a

recrystallized one.<sup>39</sup> Especially the surfaces of dentin were revealed by CO<sub>2</sub> laser irradiation when the focused energy was adequate, but sometimes too much energy causing melting led to recrystallization of the mass after cooling down, which often plugged dentinal tubules. When too much energy combined with a shortage of water supply was focused on one place, overheating denatured the organic components of dentin, leading to carbonization. The carbonization mass was weak in itself and weakly attached to intact dentin, so the openings of dentinal tubules were visible after the carbonization mass had easily been removed by a mechanical force of air or water during laser irradiation.

### Analysis of CLSM Findings

The CLSM system scans stationary samples and observes the conditions of the surface, which is similar to SEM. Because subsurface imaging is also possible, CLSM images can be reconstructed three dimensionally.<sup>40</sup> Some reports have observed CO<sub>2</sub> laser irradiated samples using CLSM.<sup>41,42</sup> Most of the surface dentinal tubules were sealed after CO<sub>2</sub> laser irradiation,<sup>22</sup> but some remained open.<sup>41</sup>

In this study, because the laser beam was not irradiated in one direction, the cavity walls in enamel and dentin were irregular and uneven, resulting in a very complicated cavity preparation. As shown in SEM findings, the cavity had many concavities on the inside wall, which could be described as micro-undercut preparation. This feature may have caused the dim outline in the CLSM images, which was not observed in 10.6- $\mu$ m CO<sub>2</sub> lasers.<sup>41,42</sup> The complicated form of the cavities was only observed three dimensionally.

It was also suggested that dentinal tubules could not be observed because they were sealed more by melting, which was confirmed by SEM. Because dentinal tubules were mostly sealed, poor penetration of dyes made the subsurface imaging less clear. Although a variety of dyes have been tried in dentin to achieve maximum imaging depth and better resolution,<sup>43-45</sup> it is necessary to develop more effective fluorescent dyes for observing 9.6- $\mu$ m CO<sub>2</sub> laser tooth tissue.

### Analysis of SEM-EDX

In this study, there was no statistically significant difference in the concentration of the mineral components in enamel surfaces, but the weight percentage of Ca and

P showed a tendency to increase in the dentin surface. This information suggested that a percentage increase of Ca or P resulted from the laser evaporation of organic components, because dentin contains much more organic material, such as collagen.

Ca:P weight ratio and Ca:P atomic ratio showed no statistically significant changes after either drilling or laser irradiation. It was suggested that there was no statistically significant change in weight and chemical elements among mineral components, while some non-mineral components were lost only in the laser group. No statistical difference was recognized in any aspect of the drilling group, which meant the turbine bur exerts only a mechanical force.

The level of P has been found to significantly decrease following irradiation with the XeCl excimer laser.<sup>46</sup> The changes in mineral content has been attributed to the effect of laser on hydroxyapatite crystals following melting and recrystallization on the dentin surfaces.<sup>47</sup> A decrease in Ca and P levels was shown with Nd:YAG laser when temperature did not increase enough to allow recrystallization, but phase changes occurred.<sup>48</sup> Conversely, the quantities of Ca or P in Er,Cr:YSGG laser areas were found to be significantly greater compared with nonirradiated areas.<sup>49</sup> The Ca:P ratio was unchanged following laser irradiation because Er,Cr:YSGG laser did not damage dentin, unlike the Nd:YAG laser. The laser irradiation did not have any effect on the mineral content of enamel and dentin.<sup>35</sup> This finding showed that 9.6- $\mu$ m CO<sub>2</sub> laser had no influence on the tissues.

### Thermal Damage to the Pulp

CO<sub>2</sub> laser energy does not penetrate deeply into tooth tissue because almost 100% of the beam is absorbed by the water-rich surface (except for scattering and reflection).<sup>36</sup> It was reported that CO<sub>2</sub> laser irradiation does not cause pulpal damage.<sup>33</sup> The short- and long-term pulpal effects of 9.6- $\mu$ m CO<sub>2</sub> laser irradiation were investigated, and there was a total absence of any degenerative changes or acute inflammatory cell response throughout the observation period.<sup>31,50</sup> Though the response of dental pulp was not investigated in this study, the thermal denatured layer on the surface was less than 1  $\mu$ m, and the tissue underneath the surface looked healthy, with no visible denaturing. Therefore, it may be inferred that the damage to the dental pulp is small. Pain induced by laser irradiation, rise in pulpal temperature, and the effect on dental pulp could not be verified because extracted teeth

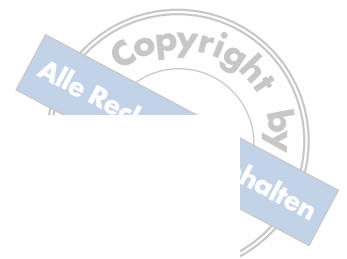
were used in this study. Therefore, further experiments on the effects on vital human pulps are warranted prior to clinical application.

## CONCLUSION

Under the parameters employed in this study, it was possible to remove enamel using 9.6- $\mu\text{m}$  CO<sub>2</sub> laser with almost no carbonization. In dentin, signs of carbonization were observed in spite of water cooling. It was thought that the spiral irradiation increased the absorption rate with less reflection, and the 9.6  $\mu\text{m}$  wavelength enabled more efficient absorption of the beam by hydroxyapatite.

## REFERENCES

- Stern RH, Sognnaes RF. Laser effect on dental hard tissues. *J South Calif Dent Assoc* 1965;33:17-19.
- Stern RH. Dentistry and the laser. In: Wolbarsht ML (ed). *Laser Applications in Medicine and Biology*, vol II, New York: Plenum Press, 1974:361-388.
- Goldman L, Gray JA, Goldman J, Goldman B, Meyer R. Effect of laser beam impacts on teeth. *J Am Dent Assoc* 1965;70:601-606.
- Taylor R, Shklar G, Roeber F. The effects of laser radiation on teeth, dental pulp, and oral mucosa of experimental animals. *Oral Surg Oral Med Oral Pathol* 1965;19:786-795.
- Koort HJ, Frentzen M. YAG-lasers in restorative dentistry. A histological investigation. *SPIE Proc* 1992;1643:403-411.
- Hibst R, Keller U. Experimental studies of the application of the Er:YAG laser on dental hard substances: I. Measurement of the ablation rate. *Lasers Surg Med* 1989;9:338-344.
- Matsumoto K, Nakamura Y, Mazeki K, Kimura Y. Clinical dental application of Er:YAG laser for class V cavity preparation. *J Clin Laser Med Surg* 1996;14:123-127.
- Kojy R. A study of microstructural changes of dental hard tissues and pulpal response after Er:YAG laser irradiation. *J Showa Univ Dent Soc* 1999;19:42-55.
- Matsumoto K, Hossain M, Tsuzuki N, Yamada Y. Morphological and compositional changes of human dentin after Er:YAG laser irradiation. *J Oral Laser Applic* 2003;3:15-20.
- Koort HJ, Frentzen M. Laser effects on dental hard tissues. In: Miserendino LJ, Pick RM (eds). *Lasers in Dentistry*. Chicago: Quintessence Publishing, 1995:57-71.
- Wang X, Matsumoto K. Pulpal response to cavity preparation by Er,Cr:YSGG laser: An in vivo study. *J Oral Laser Applic* 2005;5:23-30.
- Frame JW. Treatment of sublingual keratosis with the CO<sub>2</sub> laser. *Br Dent J* 1984;156:243-246.
- Pick RM, Pecaro BC, Silberman CJ. The laser gingivectomy. The use of the CO<sub>2</sub> laser for the removal of phenytoin hyperplasia. *J Periodontol* 1985;56:492-496.
- Nakamura Y, Yoshida T, Tsuzuki N, et al. The effect of TEA CO<sub>2</sub> laser irradiation to the rat's mandibula. *J Jpn Soc Laser Dent* 1994;5:81-90.
- Small IA, Osborn TP, Fuller T, Hussain M, Kobernick S. Observations of carbon dioxide laser and bone bur in the osteotomy of the rabbit tibia. *J Oral Surg* 1979;37:159-166.
- Meyer D-R, Scholz C, Klanke J, et al. The short pulsed CO<sub>2</sub> laser with 9.6- $\mu\text{m}$  wavelength in comparison to the erbium:YAG-laser in the laser osteotomy. *Laser Med Surg* 1990;6:150-155.
- Kantola S. Laser-induced effects on tooth structure IV. A study of changes in the calcium and phosphorus contents in dentine by electron probe microanalysis. *Acta Odont Scand* 1972;30:463-474.
- Kantola S. Laser-induced effects on tooth structure V. Electron probe microanalysis and polarized light microscopy of dental enamel. *Acta Odont Scand* 1972;30:475-484.
- Kantola S, Laine E, Tarna T. Laser-induced effects on tooth structure VI. X-ray diffraction study of dental enamel exposed to a CO<sub>2</sub> laser. *Acta Odont Scand* 1973;31:369-379.
- Kantola S. Laser-induced effects on tooth structure VII. X-ray diffraction study of dentine exposed to a CO<sub>2</sub> laser. *Acta Odont Scand* 1973;31:381-386.
- Stern RH, Vahl J, Sognnaes RF. Lased enamel: Ultrastructural observations of pulsed carbon dioxide laser effects. *J Dent Res* 1972;51:455-460.
- Nammour S, Renneboog-Squilbin C, Nyssen-Behets C. Increased resistance to artificial caries-like lesions in dentin treated with CO<sub>2</sub> laser. *Caries Res* 1992;26:170-175.
- Stewart L, Powell GL, Wright S. Hydroxyapatite attached by laser: A potential sealant for pits and fissures. *Oper Dent* 1985;10:2-5.
- Nelson DGA, Shariati M, Glana R, Shields CP, Featherstone JDB. Effect of pulsed low energy infrared laser irradiation on artificial caries-like lesion formation. *Caries Res* 1986;20:289-299.
- Cooper LF, Myers ML, Nelson DGA, Mowery AS. Shear strength of composite bonded to laser-pretreated dentin. *J Prosthet Dent* 1988;60:45-49.
- Lieberman R, Segal TH, Nordenberg D, Serebro LI. Adhesion of composite materials to enamel: Comparison between the use of acid and lasing as pretreatment. *Lasers Surg Med* 1984;4:323-327.
- Melcer J, Farcy JC, Helias G, Badiane M. Preparation of cavities using T.E.A. CO<sub>2</sub> laser. *Proceeding of the Third International Congress on Lasers in Dentistry*; Salt Lake City, UT 1992:249-250.
- Koort HJ, Frentzen M. The effect of TEA-CO<sub>2</sub>-laser on dentin. *Proceeding of the Third International Congress on Lasers in Dentistry*; Salt Lake City, UT 1992:251-252.
- Gertich K, Krautschick G, Graehn G. Anwendung eines mini-TEA-CO<sub>2</sub>-Lasers in der Zahnheilkunde. *Dtsch Stomatol* 1991;41:446-450.
- Fried D, Ragadio J, Akrivou M, Featherstone JDB. Dental hard tissue modification and removal using sealed transverse excited atmospheric-pressure lasers operating at  $\lambda=9.6\text{-}\mu\text{m}$  and  $10.6\ \mu\text{m}$ . *J Biomed Optics* 2001;6:231-238.
- Nair PNR, Baltensperger M, Luder HU, Eyrych GKH. Observations on pulpal response to carbon dioxide laser drilling of dentine in healthy human third molars. *Lasers Med Sci* 2005;19:240-247.
- Altshuler G, Grisimov V, Ermolaev V, Vityaz I. Human tooth as an optical device. *SPIE Proc* 1991;1429:95-104.
- Wigdor HA, Walsh JT. Histologic analysis of the effect on dental pulp of a 9.6- $\mu\text{m}$  CO<sub>2</sub> laser. *Lasers Surg Med* 2002;30:261-266.



34. Frentzen M, Winkelsträter C, van Benthem H, Koort H-J. The effects of pulsed ultraviolet and infra-red lasers on dental enamel. *Eur J Prosthodont Rest Dent* 1996;4:99-104.
35. Moshonov J, Stabholz A, Bar-Hielel R, Peretz B. Chemical analysis and surface morphology of enamel and dentin following 9.6  $\mu\text{m}$  CO<sub>2</sub> laser irradiation versus high speed drilling. *J Dent* 2005; 33:427-432.
36. Carruth JA, McKenzie AL. Chapter 3. The production of surgical laser lesions. In: Carruth JA, McKenzie AL (eds). *Medical Lasers: Science and Clinical Practice*. Bristol: Adam Hilger, 1986:51-80.
37. Nelson DGA, Wefel JS, Jongebloed WL, Featherstone JDB. Morphology, histology and crystallography of human dental enamel treated with pulsed low-energy infrared laser radiation. *Caries Res* 1987;21:411-426.
38. Malmström HS, McCormack SM, Fried D, Featherstone JDB. Effect of CO<sub>2</sub> laser on pulpal temperature and surface morphology: an in vitro study. *J Dent* 2001;29:521-529.
39. Matsumoto K, Hossain M, Yamada Y, Tsuzuki N. SEM and TEM findings of human dentin irradiated by Nd:YAG laser. *J Oral Laser Applic* 2001;1:195-199.
40. Watson TF. Applications of confocal scanning optical microscopy to dentistry. *Br Dent J* 1991;9:287-291.
41. Kimura Y, Wilder-Smith P, Krasieva TB, Arrastia-Jitosho AMA, Liaw L-HL, Matsumoto K, Berns MW. Visualization and quantification of healthy and carious dentin structure using confocal laser scanning microscopy. *SPIE Proc* 1996;2672:64-72.
42. Kimura Y, Wilder-Smith P, Krasieva TB, Liaw L-HL, Matsumoto K. Effects of CO<sub>2</sub> laser on human dentin: A confocal laser scanning microscopic study. *Lasers Life Sci* 1998;8:1-12.
43. Watson TF. A confocal microscopic study of some factors affecting the adaptation of a light-cured glass ionomer to tooth tissue. *J Dent Res* 1990;69:1531-1538.
44. Watson TF. Applications of high-speed confocal imaging techniques in operative dentistry. *Scanning* 1994;16:168-173.
45. Kimura Y, Takahashi-Sakai K, Wilder-Smith P, Krasieva TB, Liaw L-HL, Matsumoto K. Morphological study of the effects of CO<sub>2</sub> laser emitted at 9.3  $\mu\text{m}$  on human dentin. *J Clin Laser Med Surg* 2000;18:197-202.
46. Dankner E, Neev J, Stabholz A, Rotstein I. Effect of XeCl-308 nm excimer laser on the mineral content of human dentin. *Endod Dent Traumatol* 1997;13:234-237.
47. Stabholz A, Neev J, Liaw L-HL, Stabholz A, Khayat A, Torabinejad M. Sealing of human dentinal tubules by XeCl 308-nm excimer laser. *J Endod* 1993;19:267-271.
48. Rohanizadeh R, LeGeros RZ, Fan D, Jean A, Daculsi G. Ultrastructural properties of laser-irradiated and heat-treated dentin. *J Dent Res* 1999;78:1829-1835.
49. Hossain M, Kinoshita J-I, Yamada Y, Jahan KMR, Nakamura Y, Matsumoto K. Compositional and structural changes of human dentin following Er,Cr:YSGG laser irradiation in vitro. *J Oral Laser Applic* 2006;6:1-6.
50. Goodis HE, Fried D, Gansky S, Rechmann P, Featherstone JDB. Pulpal safety of 9.6- $\mu\text{m}$  TEA CO<sub>2</sub> laser used for caries prevention. *Lasers Surg Med* 2004;35:104-110.

**Contact address:** Dr. Kaori Nagai, Department of Clinical Cariology and Endodontology, Showa University School of Dentistry, 2-1-1 Kitasenzoku, Ohta-ku, Tokyo 145-8515, Japan. Tel: +81-3-3787-1151 (ext. 247), Fax: +81-3-3787-1229. Tel/Fax: +81-3-5702-1484 (direct). e-mail: nkaori@senzoku.showa-u.ac.jp