Compositional and Structural Changes of Human Dentin Following Er,Cr:YSGG Laser Irradiation In Vitro

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Purpose: The present study was performed to examine the morphological and compositional changes of human dentin after Er,Cr:YSGG laser irradiation in vitro.

Materials and Methods: Twenty dentin specimens were prepared and subjected to Er,Cr:YSGG laser irradiation at 3W (33.9 J/cm^2) energy density under continuous water spray (1 ml/min). For morphological studies, 5 samples were observed with SEM and 5 with TEM; diamond-knife-cut surfaces of nonirradiated areas were used as control. The remaining 10 samples were subjected to atomic analysis by SEM-EDX. Statistical analysis was performed between lased and unlased areas (control) using the Mann-Whitney U test; a value of $p < 0.01$ was considered significant.

Results: SEM observation revealed a scaly or irregular surface after Er,Cr:YSGG laser irradiation and an absence of smear layer; the orifices of dentinal tubules were exposed. TEM observation of cross sections of lased dentin showed two different zones: the uppermost surface was the ablated zone, containing irregular microparticles, and underneath this layer was an unaffected zone. On the other hand, the diamond-knife-cut surface of dentin showed no microfragment-like structures, and dentinal tubules were also intact. The results of SEM-EDX revealed no significant differences between the Ca:P ratios of lased and unlased areas.

Conclusions: Lased dentin surfaces have an etched appearance, but microfragment-like structures are also present which may hamper adhesion of composite restorative materials.

Keywords: Er,Cr:YSGG laser, human dentin, morphological change, compositional change, TEM, SEM, SEM-EDX.

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During the last several years, the potential applications of the Er:YAG[1-5] and Er,Cr:YSGG[6-7] lasers on dental hard tissue, such as carious dentin removal or cavity preparation for restorations, have been explored by a number of investigators and implemented clinically.[8-10] These lasers can ablate enamel and dentin effectively, due to their highly efficient absorption in both water and hydroxyapatite.[11] The ability of Er:YAG laser to remove enamel and dentin was found comparable to that achieved with conventional rotating instruments,[1-2] and produces minimal thermal damage to the pulp and surrounding tissues, especially when applied with continuous water spray.[3-5,12] Animal histological studies showed that pulp response to the Er:YAG laser appears to be similar to the response to high-speed handpiece application.[13] Studies on the alteration of enamel and dentin surfaces showed that Er:YAG laser is capable of decreasing the microleakage of composite resin restorations, with an effectiveness similar to that of etched, bur-prepared cavities.[14]

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.[6-7] Cavity preparation with this laser device showed that it is possible to produce shallow cavities within a few minutes; under adequate water spray, it produces minimal thermal damage to the surrounding tissues and minimal thermally induced changes of dental hard tissue composition.[16] Cutting effectiveness could also be increased when laser irradiation was applied under water spray.[7] Histological studies showed that no pulpal inflammatory responses could be identified in tissue treated with Er,Cr:YSGG laser and a water spray.[15,17] SEM studies on the surface alteration of dental hard tissues after Er,Cr:YSGG laser irradiation have been described as scaly or flaky, or as an irregular surface, and these surfaces are said to be more favorable for composite resin restorations.[6,7,16,18] However, further validation of the results of light microscopy and SEM is necessary.

The current study was conducted to investigate the morphological and compositional changes of human dentin after Er,Cr:YSGG laser irradiation by light microscopy (LM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic analysis by SEM-EDX.

MATERIALS AND METHODS

Sample preparation

Twenty extracted, noncarious human molars were used. From these teeth, 20 dentin specimens were prepared by horizontal sectioning at the middle third of the crown, and the surfaces exposed were polished with 1000-grit abrasive paper (Marumoto Kogyo, Tokyo, Japan).

Laser device and irradiation

An Er,Cr:YSGG laser system (Millennium, Biolase Technology, San Clemente, CA, USA) emitting photons at a wavelength of 2.78 µm, a pulse duration between 140 and 200 µm, and a pulse repetition rate of 20 pulses per second (20 Hz) was used. The output power of this laser device can be varied from 0 to 6 W. The beam spot size was 0.442 mm^2 with the use of a 750-µm diameter fiber at a distance of 1.5 to 2 mm. Irradiation was performed with an energy density of 3 W (33.9 J/cm^2) and 70% air/20% water in noncontact mode. Dentin specimens were exposed to laser irradiation under continuous water spray (1 ml/min).

Evaluation

After laser irradiation, all samples were observed by stereoscopy (SMZ-10, Nikon, Tokyo, Japan). Furthermore, 5 lased samples were subjected to an SEM investigation. These specimens were dehydrated in a graded series of ethanol, critical-point dried with CO_2, and mounted on aluminum stubs. Specimens were then sputter coated with platinum (thickness of 15 µm) for scanning electron microscopy (JSM-T220A, JEOL, Tokyo, Japan) at 20 kV and variable magnification.

Five lased samples were subjected to transmission electron microscopy (TEM) (H-300 & H-800, Hitachi, Japan). These samples were demineralized with EDTA, dehydrated with ethanol, embedded in epoxy resin, and then cut to 90-nm-thick sections with a diamond knife. Diamond-knife-cut surfaces of a nonirradiated area was used as control.

For atomic analysis of the remaining 10 samples by SEM-EDX, the following procedures were followed, as described in a previous study.[19] At first, cut sections of these samples were fixed with a 10% formalin solution for 48 h, then immediately perfused with a phosphate buffered solution of pH 7.3 at room temperature, and rinsed with distilled water. The samples were dehydrated in 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 using SEM-EDX (S-2500Cx, Hitachi; Model Delta V 1, Kevex, Foster City, CA, USA) at 10 kV accelerating voltage, tilt angle 350 degrees, and 3000X magnification. Statistical analyses were performed between lased and un-

Fig 1 Representative SEM photographs of dentin surface after Er,Cr:YSGG laser irradiation. Irradiation with a water mist showed a scaly appearance or an irregular surface, absence of a smear layer, and the orifices of dentinal tubule were exposed. The intertubular dentin was ablated to a greater extent than the peritubular dentin, showing a protrusion of the tubules. Peritubular dentin (arrow) appeared whitish and seemed to be thermally degenerated or recrystallized (original magnification 3500X, bar = 1.4 µm).

lased (control) areas using the Mann-Whitney U-test; a value of $p < 0.01$ was considered significant.

RESULTS

Under light microscopy, samples treated with Er,Cr:YSGG laser and water mist revealed a rough and irregular surface which appeared whitish when dried with an air syringe. Natural colors were maintained; there was no evidence of charring, carbonization, or cracking of the dentin.

SEM observation showed a scaly or irregular surface and an absence of a smear layer after Er,Cr:YSGG laser irradiation with water cooling; the orifices of dentinal tubules were exposed (Fig 1). The intertubular dentin suffered more ablation than the peritubular dentin, making the tubules protrude. In addition, peritubular dentin appeared whitish and seemed to be thermally degenerated or recrystallized (Fig 1).

TEM observation of cross-cut sections of lased dentin presented two different zones. The uppermost surface, characterized as zone of ablation, revealed irregular microfragment-like structures of 0.5 µm diameter. Underneath this structure, an unaffected zone, where intertubular dentin appeared intact, was discovered (Fig 2a). Viewing the ablation zone at 3000X magnification, the highly irregular surface was found to be a result of microexplosions due to laser irradiation; microfragment-like structures were also noted (Fig 2b). Dentin fragments were found scattered randomly over a small area; microfragment-like structures were also detected on some areas of the lased surface (Fig 2b). In contrast, nonirradiated diamond-knife-cut surfaces (control) appeared clean; no microfragment-like structures were seen and dentinal tubules were found intact (Fig 3).

The results of atomic analysis showed that the quantities of Ca (Ca weight%) and P (P weight%) were significantly greater ($p < 0.01$) in the lased area compared with the nonirradiated control areas (Table. 1). However, no significant differences were found between the Ca:P ratios of lased and unlased areas.

DISCUSSION

In the present study, when Er,Cr:YSGG laser was applied together with a water spray, neither carbonization nor melting in the lased areas or in the surrounding tissues was found. There was no evidence of charring or cracking of the dentin. These findings agree with the previous studies of Er,Cr:YSGG laser irradiation, emphasizing that minimal thermal damage to the surrounding tissues can be expected when a continuous water spray is utilized.[7] The lased dentin surface also showed micro-irregularities and an absence of a smear layer (Fig 1). These structures resembled the scaly or irregular surface of laser-irradiated surfaces in previous studies on this laser.[6,7,16,18] Furthermore, peritubular dentin appeared whitish and seemed to be thermally degenerated or recrystallized after Er,Cr:YSGG laser irradiation, which corresponded to a previous study.[20] These surfaces were more resistant to acid

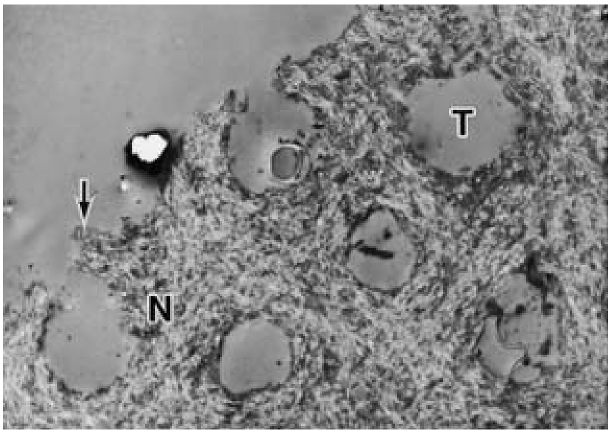

Fig 2a Representative TEM photograph of cross-cut section of dentin after Er,Cr:YSGG laser irradiation with water mist. At 3000X magnification, lased dentin presented two different zones; the uppermost surface is the ablated zone that contained irregular microparticles of 0.5 µm (arrow), beneath this structure is the normal zone (N), where dentinal tubules (T) and intertubular dentin appeared intact.

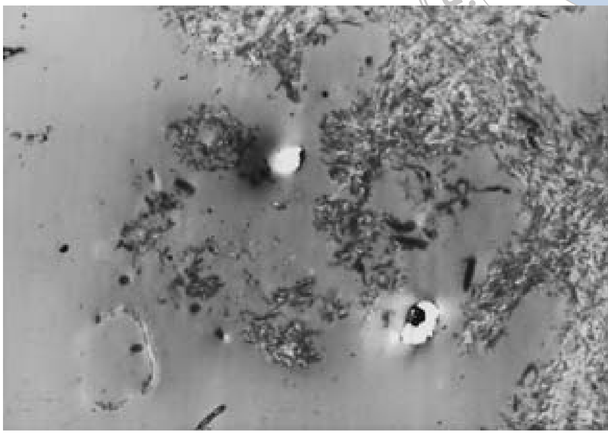

Fig 2b Representative TEM photograph of cross-cut section of dentin after Er,Cr:YSGG laser irradiation with water mist. Viewing the ablation zone at 3000X magnification, it seemed that the highly irregular surface was developed due to the microexplosion effect of the laser device; microfragment-like structures were also noted.

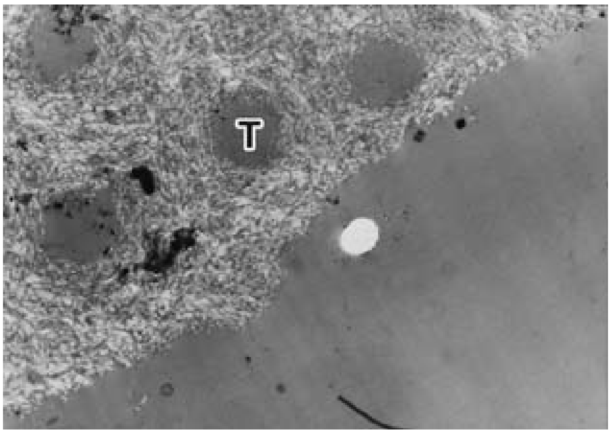

Fig 3a Representative TEM photograph of cross-cut section of nonirradiated diamond-knife-cut surface of dentin. At 3000X magnification, microfragment-like structures were not seen and dentinal tubules (T) were intact.

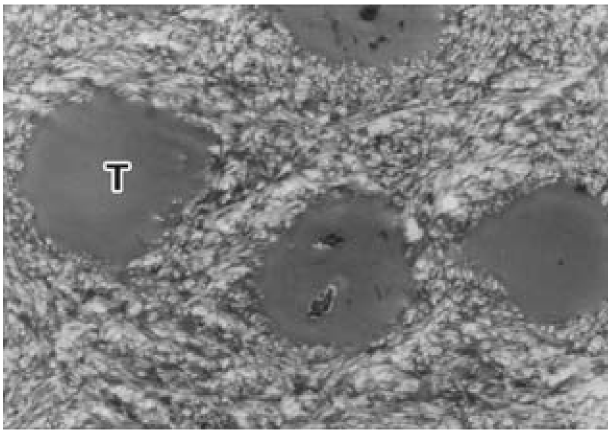

Fig 3b Representative TEM photograph of cross-cut section of nonirradiated diamond-knife-cut surface of dentin. At 5000X magnification, dentinal tubules (T) appeared intact.

dissolution than the unlased areas and are thought to have a caries-preventive effect.[20]

The TEM analysis of the dentin surfaces after irradiation validates the results of light microscopy and SEM. When the zone of ablation on the surface was investigated at high magnification, a highly irregular surface was found (as seen in SEM), which had developed due to the micro-explosions caused by the laser. This is the dominant mechanism of hard tissue ablation with the Er:YAG and Er,Cr:YSGG lasers.[11] The Er,Cr:YSGG laser has high absorption in water due to its wavelength. The conversion of radiation into thermal energy causes a local concentration of heat, and when the water is heated to boiling point, an explosive vaporization will occur. It is therefore termed a hydrokinetic system.[10]

Immediately underneath the zone of ablation, an unaffected zone was discovered, where intertubular

Table 1 Results of atomic analysis by SEM-EDX (mean ± SD)

	Lased area	Nonirradiated area
Ca (weight%)	34.14 ± 2.86[a]	26.26 ± 2.55[a]
P (weight%)	16.75 ± 2.41[b]	12.85 ± 2.68[b]
Ca/P	2.03	2.04

[a, b] Shows a significant difference ($p<0.01$)

dentin appeared intact (Fig 2a). There was no apparent ablation of the mineral zone. Considering the minimal thermal effect on underlying mineral components, it can be assumed that micro-explosions were limited to the superficial area, not penetrating into the deep intact zone. In contrast to the Er,Cr:YSGG laser, an ablation of mineral components was apparent with the Er:YAG laser in our previous study.[21] The ablation of mineral components in this case may be due to a thermal effect, where water mist is used predominantly as a coolant.

SEM-EDX examination revealed that the quantities of Ca or P in the lased areas were significantly greater compared with nonirradiated areas. It is likely that during the laser irradiation, a percent increase of Ca or P results from the evaporation of organic components. These changes are possibly a thermal side effect of Er,Cr:YSGG laser irradiation. However, the Ca:P ratio was unchanged following laser irradiation, as also found in a previous study of cavity preparation with the Er,Cr:YSGG laser.[16] Minimal thermal changes of dentin components after Er,Cr:YSGG laser irradiation with a water mist could also be achieved. Such a phenomenon was not seen with the Nd:YAG laser when applied at a high energy density; the Ca:P ratio was low compared to that of the nonirradiated dentin.[22]

Bonding resin composites to dental hard tissues is one of the most significant contributions to restorative dentistry.[23] Initially to enamel,[24] and afterwards to dentin,[25] the micromechanical retention of resin-based materials inside the porosities created by acid etching dental hard tissues is currently the most successful method of dental bonding.[23,26,27] However, with acid etching (using phosphoric acid), chemical changes may produce an increase in dentin permeability and in dentin wetness, increasing the potential for pulp irritation, modification of the proportion of organic matter, and decalcification of the inorganic component.[28,29]

As a possible alternative to the acid-etching technique, the use of laser shows promise from current research. Laser application yields an effect similar to etching with high roughness values and open dentinal tubules, without damage to the underlying tissues and dental pulp.[6,7,15-18] Moreover, laser therapy facilitates or even improves bond strength.[31] Therefore, it was thought that laser etching could replace acid etching. However, the present TEM study revealed that some microfragment-like structures were also found scattered over small areas of the Er,Cr:YSGG lased surface. Nonirradiated control areas (diamond-knife-cut surfaces), on the other hand, appeared clean; no microfragments were seen. Fragments shown by the TEM in lased areas might have an adverse effect on the adhesion of composite materials, a question which must be answered before clinically using this laser irradiation technique for enamel and dentin etching.

CONCLUSIONS

Lased dentin surfaces reveal an etching pattern, but they are also associated with microfragment-like structures which might have an adverse effect on the adhesion of composite restorative materials. Therefore, it is still too early to maintain that structural changes in dentin following laser irradiation are favorable to the adhesion of composite restorative materials in the clinic situation.

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