Cavity preparation with conventional low- and high-speed handpieces involves irritating noise, uncomfortable vibrations, stress, and pain for patients. These disadvantages have led to continuous research for alternatives in dental hard tissue removal. The first lasers used in dentistry were CO2 and Nd:YAG. However, some problems resulted from side effects which may cause surface changes, such as roughness, cratering, cracking, fissuring, and melting. In addition, since tissue interaction with these lasers is photothermal, the profound thermal effect leading to pulpal damage and the inability to precisely cut biocalcified tissues have limited the use of such lasers in clinical practice.

Recent developments have led to a new generation of laser devices, such as Er,Cr:YSGG laser and Er:YAG laser, with improved efficacy and reduced heat distribution for application in hard tissues. Er,Cr: YSGG laser is one of the most promising among them. The 2.78 μm wavelength allows it to be more or less completely absorbed by water molecules in enamel and dentin. Some preliminary studies have reported that this laser can provide clinical procedures with minimal patient discomfort in an adequate preparation time, without any mutagenic or carcinogenic effects. However, before clinical application of this system in patients, safety issues and pulpal effects should be carefully evaluated in animal models.

The purpose of this study was to investigate the short and long-term pulpal effects of Er,Cr: YSGG laser cavity preparation in coronal dentin in beagle dogs.
MATERIALS AND METHODS

Laser Device

An Er,Cr:YSGG laser device (Millennium, Biolase, San Clemente, CA, USA) with a 2.78 µm wavelength, pulse duration from 140 to 200 µs, and a repetition rate of 20 Hz was employed throughout the study. The power output can be varied from 0 to 6 W. The delivery system consisted of a fiber-optic tube terminating in a handpiece with a sapphire crystal tip bathed in an adjustable air-water spray. The beam spot size was 1.26 x 10^-3 mm² (tip diameter: 400 µm).

Experimental Animals

Permission was obtained from the Committee of Animal Experimentation of Showa University, Japan, to use 5 male beagle dogs, 10 to 12 months of age and approximately 10.5 to 12.1 kg in weight. The animals were kept individually in metal cages at room temperature, 12 h light per day, and 40% relative humidity. They were fed a combination of hard/dry and soft/moist diet throughout the study. Following standard surgical procedure, food and water were withheld for 12 hours prior to surgery. A total of 113 teeth (incisors, canines, and molars) were used in a split mouth clinical design: all animals, quadrants, different tooth types, and treatments were represented at each time interval. The teeth were systematically assigned so that each treatment group was represented by 5 or 6 teeth, which were from different quadrants in different animals at different treatment periods. Four untreated teeth served as controls. Treatment periods were arranged so that histological observation could be performed at 0, 3, 7, 30, 90 days after cavity preparation. The distribution of the teeth used in the experiment is presented in Tables 2 to 4.

Surgical Procedure

General anesthesia was obtained using intravenous injections of 6% pentobarbital, 20 mg/kg for induction and a 1/10 to 1/5 dose for maintenance in the animals. The teeth were treated with either Er,Cr:YSGG laser or a tapered diamond bur (ISO #013). Laser irradiation was performed in contact mode, with the sapphire tip kept perpendicular to the irradiated enamel surface. The output was 4.0 W with a 70% water spray and a 70% air spray (water supply: 25 ml/min). Energy density was 160 J/cm². Preparation with a bur was performed with a PXN930 unit (Yoshida, Tokyo, Japan) and a high-speed diamond bur at 20,000 rpm in copious water spray. All surgical cutting was performed by the same operator, and a Class V cavity was prepared in the buccal (labial) surface of each tooth. Each cavity was about 4 mm in diameter at the enamel surface. The depth of the cavity was verified visually: approximately one-third of the distance (1/3 DEP) from the enamel surface to the pulp (0.67 to 1.0 mm), or two-thirds the distance (2/3 DEP) from the enamel surface to the pulp (1.33 to 1.67 mm). Thereafter, equal lengths of base and catalyst of Dycal (Caulk Division, Dentsply International, Milford, DE, USA) were mixed, a thin layer was placed on the exposed dentin, and the cavities were filled with amalgam. The dogs were subsequently returned to their cages.

Table 1 Pulpal response scoring system

<table>
<thead>
<tr>
<th>Score</th>
<th>Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal pulp: no observable difference compared with control (Fig 1)</td>
</tr>
<tr>
<td>1</td>
<td>Mild reaction: edema, slight disruption of odontoblastic layer, vascular dilation, hyperemia, hemorrhage, occasional interstitial inflammation cells (Fig 2)</td>
</tr>
<tr>
<td>2</td>
<td>Moderate reaction: necrosis in odontoblastic layer, edema, hyperemia, vascular dilation, hemorrhage, increased number of inflammation cells (Fig 3)</td>
</tr>
<tr>
<td>3</td>
<td>Severe reaction: generalized necrosis of pulp elements, edema, vascular dilation, hemorrhage, increased number of inflammation cells (Fig 4)</td>
</tr>
</tbody>
</table>
Fig 1 Score 0 (normal pulp). No significant alterations in pulp structure are noted immediately after Er,Cr:YSGG laser cavity preparation. H-E stain, original magnification 100X.

Fig 2 Score 1 (mild reaction). The odontoblastic layer shows mild disruption. Vascular dilation, hyperemia, and edema are noted. Three days after Er,Cr:YSGG laser cavity preparation. H-E stain, original magnification 100X.

Fig 3 Score 2 (moderate reaction). Disruption, localized necrosis of odontoblastic layer, infiltration of inflammatory cells, hemorrhage, and degeneration of pulp are observed. Thirty days after Er,Cr:YSGG laser cavity preparation. H-E stain, original magnification 60X.

Fig 4 Score 3 (severe reaction). Generalized necrosis of pulp elements, degeneration, edema, vascular dilation are observed. Thirty days after Er,Cr:YSGG laser cavity preparation. H-E stain, original magnification 100X.

Table 2  Number of experimental teeth and remaining dentin thickness (RDT) in experimental groups

<table>
<thead>
<tr>
<th>Cavity depth</th>
<th>Er,Cr:YSGG laser</th>
<th>Bur</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>RDT</td>
</tr>
<tr>
<td>1/3 DEP</td>
<td>27</td>
<td>1.05 ± 0.32</td>
</tr>
<tr>
<td>2/3 DEP</td>
<td>29</td>
<td>0.61 ± 0.38</td>
</tr>
</tbody>
</table>

Mean (mm) ± SD. n: number of experimental teeth. There was no significant difference in RDT between the laser group and the bur group (p > 0.05, Mann-Whitney U test).
Histological Observation

At the predetermined time point, the dogs were killed by means of an overdose of pentobarbital. Following death, the dogs’ maxillae and mandibles, containing the test teeth and surrounding alveolar bone, were excised and fixed in 10% neutral buffered formalin. Then samples were decalcified with Plank and Rychlo solution and embedded in selloidin. The serial, continuous histological sections (20 µm) were stained with hematoxylin and eosin, and then examined under a light microscope by three different evaluators, independently and blindly. To ensure that all evaluators followed the same evaluation protocol, they completed a preliminary training session. The pulpal response was histologically evaluated based on a scale of 0 to 3 (Table 1).19 The distance between the cavity floor and the pulp was measured with ocular and objective micrometers (Nikon, Tokyo, Japan), and the shortest distance was designated as remaining dentin thickness (RDT). Average RDT and presence or absence of tertiary dentin was also recorded during the histological examination.

Statistical Analysis

The Mann-Whitney U test was used to determine the difference between the laser and the bur groups, in av-

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**Table 3 Histological evaluation of pulpal response for 1/3 DEP**

<table>
<thead>
<tr>
<th>Period</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Total</th>
<th>0</th>
<th>1</th>
<th>2</th>
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<td>0</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>3 days</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>0</td>
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<td>0</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>30 days</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>90 days</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>0</td>
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<td>5</td>
</tr>
</tbody>
</table>

*Significant difference (p < 0.01)*

**Table 4 Histological evaluation of pulpal response for 2/3 DEP**

<table>
<thead>
<tr>
<th>Period</th>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>Total</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
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<td>2</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>3 days</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>7 days</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>30 days</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>90 days</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

*Significant difference (p < 0.01)*

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Fig 5 Mandibular anterior teeth immediately after Er,Cr:YSGG laser preparation. The margin of the cavity was sharp, and no carbonization was visible.
average RDT and mean histological score. The difference in presence of tertiary dentin between the laser and the bur groups was compared using the chi-square test. Significance was set at $p < 0.01$.

RESULTS

Because of pulp exposure or loss of restorative materials, six prepared teeth were discarded from histological evaluation. Therefore, the evaluated specimens comprised 56 teeth prepared with Er,Cr:YSGG laser and 51 teeth prepared with a high-speed dental bur. No significant difference in RDT was found between the laser groups and the bur groups when averaged over time (Table 2).

No obvious fractures or dentin cracking were observed in any of the specimens prepared with Er,Cr:YSGG laser. The margin of the cavity was sharp, and no visible carbonization was found (Fig 5). When cavities were prepared to 1/3 DEP, some slight vessel dilation and hyperemia was observed in the bur groups immediately after preparation. At 3 days, the pulp of all specimens appeared normal or showed a mild reaction: vessel dilation and slight disruption of the odontoblastic layer were observed. After 7 days, the pulp of most specimens in both groups had regained a normal appearance. When cavities were prepared to 2/3 DEP, vessel dilation and hyperemia were often found immediately after preparation, especially in the bur groups. At 3 days, some specimens in both groups exhibited degeneration or local necrosis of odontoblastic cells. After 7 days, the pulp of most specimens in both groups had recovered fully or showed only a mild reaction. When the experiment ended at 90 days, most of the pulps in both groups appeared normal. Generally speaking, cavity preparation with a high-speed bur was more often observed to cause vessel dilation and hyperemia, which tended to last for a longer period than when Er,Cr:YSGG laser was used, while Er,Cr:YSGG laser tended to cause more fibroblast cell proliferation than did bur preparation, especially at 7 days after cavity preparation (Fig 6).

The histological scores for pulpal response are shown in Tables 3 and 4. No significant difference was found between the laser group and the bur group at any time point, at cavity depths either 1/3 or 2/3 DEP ($p > 0.05$).

Tertiary dentin formation was observed in both groups on the pulp aspect of the cavity floor at 30 and 90 days after cavity preparation (Fig 7). The presence of tertiary dentin in the laser group was significantly more frequent than in the bur group ($p < 0.01$). In terms of cavity depth, tertiary dentin was significantly more frequently observed in deeper cavities (2/3 vs 1/3 DEP) (Table 5, $p < 0.01$).

DISCUSSION

One of the most essential requirements of a laser device for cavity preparation in vital teeth is its ability to...
preserve the structural and functional integrity of the dental pulp.\textsuperscript{20} Previous studies have stated that Er,Cr:YSGG laser might be a suitable tool for ideal removal of calcified dental tissues.\textsuperscript{21,22} However, there seems to be no animal study on the long-term structural effects on dental pulp after cavity preparation by Er,Cr:YSGG laser. Therefore, the present study was designed to investigate the pulpal response to Er,Cr:YSGG laser cavity preparation of teeth to different depths at different time intervals. The results showed that with a water-air cooling system and the proper settings, this laser was gentle on the pulp during the observation periods.

RDT has been considered a major indicator of the pulp response after cavity preparation.\textsuperscript{23} In order to maintain RDT of the specimens, a preliminary study was performed before conducting the present study, proving the laser parameters to be optimal for both preparing a cavity in a dog’s tooth and easily controlling the cavity depth. During the experiment, the diamond bur was marked so that the correct cavity depth would be obtained in the bur group. Specimens that showed loss of restorative materials or pulp exposure were excluded from evaluation. Dental amalgam was used for cavity restoration because the preliminary study showed the loss of restorative material to be less with amalgam than with some other materials (composite resin or glass-ionomer cement) under the described experimental conditions.

Since no statistically significant difference was found between the RDTs in the laser irradiation and high-speed bur groups, a histopathological examination was performed. Blinded assessment indicated that there was no marked difference in pulp histology at any time point, either for shallow (1/3 DEP) or deep cavities (2/3 DEP), except for the formation of tertiary dentin. This result supported the previous findings of Marx and Hof,\textsuperscript{18} Matsumoto et al,\textsuperscript{17} and Eversole et al.\textsuperscript{21} These investigators concluded that Er,Cr:YSGG laser is

![Fig 7a](image1) Thirty days after Er,Cr:YSGG laser cavity preparation. Tertiary dentin formation is noted. H-E stain. Original magnification 60X.

![Fig 7b](image2) Thirty days after Er,Cr:YSGG laser cavity preparation. Tertiary dentin formation is noted. H-E stain. Original magnification 200X. ND: normal dentin; TD: tertiary dentin; P: pulp.

<table>
<thead>
<tr>
<th>Cavity depth</th>
<th>Er,Cr:YSGG laser</th>
<th>Bur</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/3 DEP</td>
<td>3 (11)</td>
<td>0 (10)</td>
<td>3 (21) \textsuperscript{b}</td>
</tr>
<tr>
<td>2/3 DEP</td>
<td>7 (12)</td>
<td>2 (10)</td>
<td>9 (22) \textsuperscript{a}</td>
</tr>
<tr>
<td>Total</td>
<td>10 (23) \textsuperscript{a}</td>
<td>2 (20) \textsuperscript{b}</td>
<td>12 (43)</td>
</tr>
</tbody>
</table>

Number of specimens showing tertiary dentin formation (whole number of specimens in the group). Different superscript letters indicated statistically significant difference (\( p < 0.01 \)).
effective in ablation of enamel and dentin, while the operative pain is reduced. In the present study, after cavity preparation by Er,Cr:YSGG laser, no obvious fracture or structural injury of dentin was observed. The margin of the cavity was sharp, and no visible carbonization was noted. In shallow cavities (1/3 DEP), all the specimens revealed a normal pulp or mild reaction; in deep cavities (2/3 DEP), most of the specimens revealed a normal pulp or mild reaction, and at the end of this experiment, all specimens but one had recovered a normal pulp. This confirmed previous reports on the pulpal effects of Er,Cr:YSGG laser in dogs, rabbits, and humans. The minimal thermal damage to the pulp was most likely due to the wavelength of this laser, the characteristic interaction between the Er,Cr:YSGG laser and hard dental tissues, the special energy setting, and the provision of water-air cooling. The wavelength of Er,Cr:YSGG laser almost coincides with the major absorption band of water, which is present in the enamel and dentin of teeth in vivo. Among the currently used lasers that emit in the near- and mid-infrared spectral ranges, this laser’s absorbability in water is one of the greatest, approximately 10 times higher than that of CO2 laser, 200 times greater than that of Ho:YSGG laser, and 20,000 times higher than that of Nd:YAG laser. It also shows fairly high absorbability in hydroxyapatite. Moreover, the water-air cooling system also helped to reduce the heat distribution into pulp, so that the temperature rise in pulp would not reach more than 5.5°C, which is known to be the critical level for damaging dental pulp.

In the present study, two pulps were damaged beyond repair due to cavity preparation: one by Er,Cr:YSGG laser and the other by high-speed bur, and both with cavity depths of 2/3 DEP. Histopathological examination indicated that in both cases, the cavities were so deep that RDT was less than 0.15 mm. On the other hand, these two teeth were located in the dorsal mandibular molar region. It is possible that the water spray did not adequately reach this area during preparation. Therefore, caution should be taken when using Er,Cr:YSGG laser in deep cavity preparation, and copious water cooling is essential.

Although no significant difference was found in the histological scores for pulpal response between Er,Cr:YSGG laser and high-speed bur, some tendencies were noticed after cavity preparation. Vascular dilation and hyperemia were more frequent and tended to last for a longer period after bur drilling. This difference might be explained by the temperature rise in pulp cavity during cavity preparation. It was reported in a comparative study that pulpal temperature changed less than 2°C with Er,Cr:YSGG laser preparation, yet bur preparation resulted in a temperature rise as high as 4°C. After preparation, the damaged odontoblast layer was replaced by proliferative fibroblasts, which are said to play a role in the formation of tertiary dentin. Tertiary dentin formation may be seen as a reparative or defensive reaction to mild alteration in the pulp associated with cavity preparation, because tertiary dentin in 2/3 DEP cavities occurred more frequently than in 1/3 DEP cavities in the present study. We also observed earlier fibroblast proliferation and more frequent tertiary dentin formation in the Er,Cr:YSGG laser group than in the high-speed bur group, which suggests that Er,Cr:YSGG laser irradiation facilitates the healing of damaged pulp tissue more effectively than does high-speed bur preparation. Keller and Hibst reported similar findings after cavity preparation by Er:YAG laser in dogs. They described the formation of tertiary dentin in some specimens 3 to 5 weeks after Er:YAG laser preparation. This was very similar to our results 30 days after using Er,Cr:YSGG laser irradiation for cavity preparation. Widgor et al reported that the formation of tertiary dentin appeared as early as 4 days after cavity preparation with Er:YAG laser in dogs. However, no sign of tertiary dentin formation was found at 0 to 7 days postpreparation in the present study. Possible causes for the difference include the different wavelength, parameters of laser irradiation, or experimental tooth site.

Laser energy was introduced as a possible replacement for the mechanical handpiece to remove dental hard tissues in the early 1960s. Since then, many different lasers at different wavelengths have been studied. At present, Er,Cr:YSGG laser is considered one of the most promising lasers for hard tissue ablation. Potential advantages of the application of this laser for cavity preparation include: precise removal of tooth structure, reduced noise and vibration, creation of a surface morphology that enhances the bonding of resin restorations, and reduction of the permeability of dental hard tissues resulting in increased acid resistance. In addition, the encouraging results of the present study gave an indication that Er,Cr:YSGG laser is potentially a valid and safe tool for cavity preparation to the depth where RDT was as thin as 0.56 mm. However, because this study was performed using a particular energy setting, future investigations of the effects of this laser on pulp should involve pulpal response when laser irradiation and water-air cooling are set differently, and when cavities are deeper or the pulp is exposed.
CONCLUSION

Within the limitations of the present study, it can be concluded that ablation of Er, Cr:YSGG laser with adequate water-air cooling reduces deleterious thermal effects on the dental pulp. Er, Cr:YSGG laser is effective and safe in the ablation of dental hard tissue in vivo. Reaction of dental pulp was similar to the response aroused by the high-speed bur, which was generally mild and localized.

REFERENCES

25. Bayly JG, Kartha VB, Stevens WH. The absorption spectra of liquid phase H2O, HDO and D2O from 0.7 É m to 10 É m. Infrared Physics 1963;3:211-223.