〔Thesis〕 松本歯学 25：141～165，1999
key words：Er：YAG laser — Effects of irradiation — Histopathological observation

Application of Er : YAG Laser Irradiation to Immature Teeth

AKEMI KAWABATA, HIROSHI IWASAKI, ANGELI YAGASAKI and HIROO MIYAZAWA

Department of Pediatric Dentistry, Matsumoto Dental University School of Dentistry
(Chief : Prof. H. Miyazawa)

Summary

To evaluate the usefulness and safety of Er : YAG laser for cavity preparation of immature teeth, we irradiated the tooth substance using Er : YAG laser in teeth of rats in the growth stage and human immature premolars showing an incomplete root and determined appropriate irradiation conditions, observed the irradiated surface by scanning electron microscopy (SEM), evaluated the thermal effects of irradiation, and performed histopathological examination.

SEM of the irradiated surface revealed no smear layer or dentinal plug observed after cavity preparation using an air turbine, and the dentinal tubules were open. Neither carbonization nor cracks were observed after irradiation at 50, 100, or 150mJ/pulse. Evaluation of thermal effects showed a mean temperature of less than 5℃ in the dentin on the pulp side after irradiation by each combination of the following conditions : dentin thickness, 1.0 or 1.5mm irradiation time, 2, 5, or 10 seconds ; and energy, 50, 100, or 150mJ/pulse. Histopathological examination showed circulatory impairment and disarrangement of odontoblasts immediately after irradiation but tendency to recovery after 1 week.

These results suggest the applicability of YAG laser to irradiation of the tooth substance. Considering the effects on the pulp, irradiation at 50–100mJ/pulse may be useful and safe for irradiation of the tooth substance of immature teeth.

Introduction

In a previous study, we tested laser usage in cutting hard tissues by irradiating dentin using various types of laser, and we found that pulp injury, carbonization and generation of cracks caused by the heat can be inhibited by applying irradiation under optimal conditions for each purpose[1]. However, high energy with high generating power is required to cut teeth with a CO_2 laser or Nd : YAG laser. Thus, considerable heat is generated, causing evaporation of liquid. Therefore, it was thought to be difficult to irradiate to dentin, which is adjacent to the pulp.

Recently, the Er : YAG laser with a wave length of 2.94μm that causes evaporation of liquid within teeth was developed. This laser is characterized by its high adsorbability into water[2], and when this laser is applied to teeth, water molecules contained in enamel and dentin absorb energy

(submitted August 5, 1999 ; accepted November 24, 1999)

from laser. The subsequent water evaporation results in changes in volume, which are thought to be the mechanism of irradiation[2,3].

At present, air turbine handpieces are widely used to cut teeth, but since they are rotary instruments, pulpitis may be caused by the heat or vibration generated during preparation. There are also other problems to be solved including noise due to rotation, which is peculiar to air turbine handpieces. Thus, the Er : YAG laser has attracted attention as a substitute for air turbine handpieces[4]. The Er : YAG laser has the following advantages : it does not produce vibration during cutting, it can be used without anesthesia ; it may be suitable for adhesive restorations with resin or ionomer since laser etching occurs on the irradiated surfaces ; and it does not produce noise the way air turbine instruments do. Since the peculiar noise produced by air turbines causes strong anxiety and fear in children[5], laser may be used efficiently in clinical practice of pediatric dentistry if such noise can be avoided. However, little work has been done to study the application of Er : YAG lasers to deciduous teeth or erupting permanent teeth, and the safety of its clinical application has not been established.

The objective of the present study was to investigate the effectiveness and safety of Er : YAG laser application to immature permanent teeth which present peculiar physical and anatomical properties. Thus, the morphology of the cut surface was observed with a scanning electron microscope (SEM), and the effects of heat produced during irradiation on the pulp and the effects of irradiation on the pulp were examined histologically.

Materials and Methods

1. Laser equipment

An Erbium-yttrium-alminum-garnet (Er : YAG) laser ML22 (developed jointly by HOYA and J. Morita Co.) was used in this study. For irradiation, a contact tip with a diameter of 0.6mm was used.

2. Methods

1) Experiment 1 : SEM observation of the cut surfaces after laser irradiation

Male Wistar rats at three weeks of age weighing 30 to 50g were used in this experiment.

Following inhalation of diethyl ether, rats were intraperitoneally injected with 25mg/kg of thiopental sodium (Rabonal®, Tanabe Pharm. Co.). The rats were then placed on a bed specially designed for this experiment.

Irradiation was applied to the labial surface of the upper incisor on the right side with the point of the tip being in contact perpendicularly with the labial surface from the labial side to the palatal side. During irradiation, water was supplied at 25ml/min, and was vacuumed simultaneously.

The conditions of laser irradiation are shown in Table 1. A total of 15 rats were used with 5 rats used for each condition of irradiation.

After irradiation, the rats were sacrificed with an overdose of diethyl ether before a jaw block including the experimental tooth was removed. The specimens were then immersed in 10% of neutral buffered formalin solution for 7 days for fixation, and dehydration was performed using series of alco-

Table 1 Irradiation condition

Energy per pulse (mJ/pulse)	Pulse per second (pps)	energy per density (J/cm^2)
50	10	17.7
100	10	35.4
150	10	53.1

hol with increasing concentrations, according to the conventional method. After the specimen was dried, gold vapor deposition was performed, and the cut surface was observed at an acceleration voltage of 12kV with a scanning electron microscope (JEOL JCXA-733).

2) Experiment 2 : Effects of heat generated by irradiation

(1) Procedure

a. Specimen preparation

Among premolar teeth extracted for orthodontic treatment, the ones with incomplete apices that were judged immature clinically were selected for this experiment. In preparing specimens, enamel of the occlusal surface including the cusps was reduced perpendicularly to the tooth axis until dentin was exposed and the roots were removed at the level of the anatomical cervical line using an air turbine handpiece (#611 diamond bur). The exposed dentin of the occlusal surface was polished with wet abrasive paper (#800) until smooth, then was irradiated. Furthermore, a hole with a diameter of 0.5mm and a depth of 1.0mm was made in the center of the surface facing the irradiated surface, and it was used as the site of thermal cup fixation. Specimens were prepared so that the distance between the irradiated surface and the thermocouple was 1.0mm and 1.5mm. The specimens were immersed in artificial saliva at 37℃ just before the experiment.

b. Condition of irradiation

As in Experiment 1, irradiation energy was 50mJ/pulse, 100mJ/pulse and 150mJ/pulse, and the pulse rate was set at 10 pps. Irradiation time was 2s, 5s and 10s. Irradiation was performed while water of 16℃ was supplied at 25ml/min.

c. Method

A sponge was placed in a dish, and an alumel-chromel thermocouple was fixed on the sponge. After a specimen was placed, artificial saliva (Salivate®, Teijin Co.) that had been stored at 37℃ was added until the sponge was soaked.

Next, a straight handpiece was fixed with clamps. After the specimen and laser were fixed, irradiation was performed only after thermocouple stabilization was confirmed. The room temperature at the time of the experiment was 27℃. In this experiment, 5 specimens were irradiated under each condition of irradiation (Fig.1).

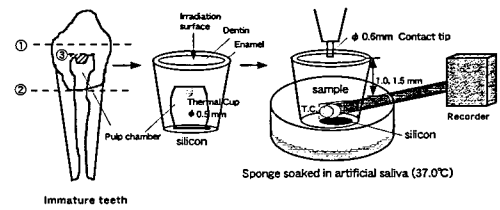


Fig.1 Methods

d. Observation method

Temperature changes were recorded before, at the time of, and after laser irradiation using a recorder (Intelligent recorder R310, Techno Seven) which was connected with an alumel-chromel thermocouple, and the mean value of 5 specimens was determined for each condition.

3) Experiment 3 : Histopathological observation

As in experiment 1, diethyl ether was used before rats were anesthetized with 25mg/kg of thiopental sodium (Rabonal®, Tanabe Pharm. Co.) intraperitoneally. Rats were then placed on the bed, and the head and body were fixed to the bed. The mouth was opened by placing power chains around the upper and lower incisors while carefully avoiding injuring the lower incisors

and the surrounding mucosa. The site of operation was cleaned.

a. Control group

To observe physiological changes and changes with time due to aging, the lower first and second molars on the left side of the same rat were selected as controls.

b. Laser irradiation group

Laser irradiation was performed on the occlusal surfaces of the lower first and second molars on the right side. The laser tip was inserted in the mouth from the occlusal surface toward the apex, then laser was irradiated with the point of the tip being perpendicularly in contact with the occlusal surface while water was being supplied at a rate of 25ml/min. Water was removed with a vacuum tip, which was also used to depress the buccal mucosa, and the tongue was moved with a tongue depressor to permit direct vision of the occlusal surfaces during irradiation. There were three conditions of irradiation as in experiment 1. The irradiation surface was blow dried before glass ionomer cement (Fuji Ionomer® Type II, GC Co.) was used. Glass ionomer was selected because of its low irritancy to the pulp, physical properties and excellent marginal seal[6,7]. The observation was performed immediately, 7 days and 30 days after irradiation.

A total of 72 rats were used in this experiment, with 8 rats used for each condition of irradiation for each observation period (Table 2).

Table 2 The group of rats

Unit : rats

Energy per pulse (mJ/pulse)	The period of experiments	
50	Immediately	8
	7 days after irradiation	8
	30 days after irradiation	8
100	Immediately	8
	7 days after irradiation	8
	30 days after irradiation	8
150	Immediately	8
	7 days after irradiation	8
	30 days after irradiation	8

(2) Specimen preparation and observation methods

After each observation period, the specimens were fixed in formalin as in experiment 1, and were demineralized using hydrochloride demineralizing agent (K-CX demineralizing agent®, Fujisawa Pharm. Co.). After completion of demineralization, the specimens were neutralized with 5% sodium sulfate and were washed with water before dehydrated in series of alcohol bath of increasing concentration. Next, the specimens were embedded in paraffin before serial section was performed at about 5 μm intervals. Then, hematoxylin-eosin staining(H-E stain) and Masson trichrome staining were performed before histopathological observation with a light microscope.

As shown in Table 3, the criteria for

Table 3 Evaluation of histopathological findings

① Hyperemia	
② Hemorrhage	
③ Inflammatory cell infiltration	
④ Irregularity, atrophy, disappearance, vacuolization of odontoblast layer	− : No mark change
⑤ Disappearance of pulpodentinal membrane	± : Immediately below irradiation surface
⑥ Rod spaped body in dentinal tuble	+ : Coronal pulp
⑦ Degeneration, necrosis of pulp cell proper	+ + : Entire pulp
⑧ Formation of osteodentin	
⑨ Proliferation of pulp cell proper	
⑩ Fibrosis	

histopathological evaluation were as follows : hyperemia and hemorrhage of the pulp tissue ; inflammatory cell infiltration ; disarray in the arrangement ; atrophy, disappearance, and vacuolation of odontoblasts ; disappearance of the pulpo–membrane ; aspiration of the nuclei of odontoblasts or erythrocytes ; necrobiosis of pulp cells ; formation of osteodentin ; hyperplasia of pulp cells proper ; and fibrosis.

In addition, to determine the minimal thickness of the remaining dentin after irradiation for each condition of irradiation, measurement was performed on the photographs of the specimens using the photo of the scale taken at the same condition as the specimens.

Results

1) Experiment 1 : SEM observation of the irradiated surface Observation of the irradiated surface at 50mJ/pulse revealed that the cavity walls were rough due to the presence of enamel rods, and the border between the irradiated surface and the non–irradiated surface was clear (Fig.2).

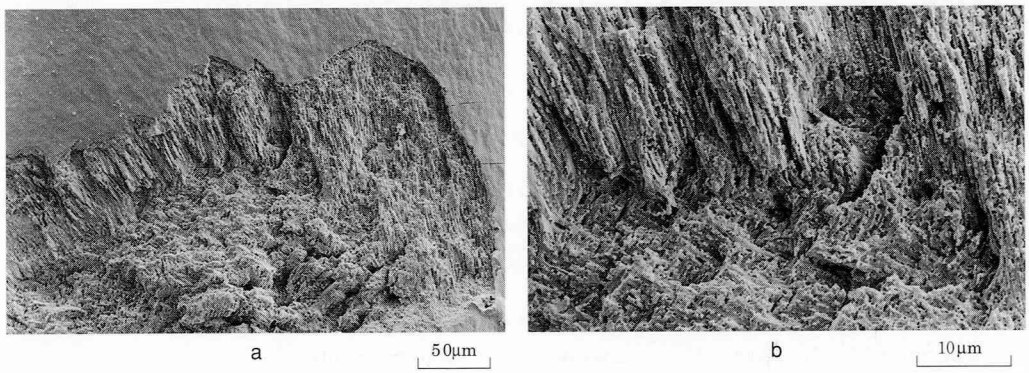


Fig.2 SEM photograph

The tooth surfaces cut with Er : YAG laser displayed irregularities by enamel rods.
a : Irradiation condition 50mJ/pulse 10pps
b : Higher magnification of Fig. 2a

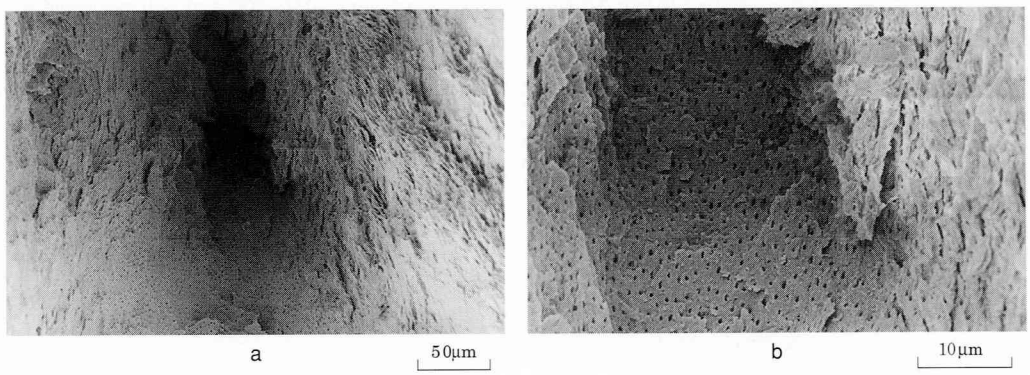


Fig.3 SEM photograph

Smear layer was not seen at Er : YAG laser irradiated dentin, and opening of the dentinal tubules were clearly observed.
a : Irradiation condition 100mJ/pulse 10pps
b : Higher magnification of Fig. 3a

Observation of the irradiated surface at 100mJ/pulse revealed that cavities were deeper than those prepared by 50mJ/pulse irradiation, and odontoblasts were open at the wall without smear layer. The deepest part of the cavity at higher magnification showed a comparatively flat floor (Fig.3).

When the irradiated surface by 150mJ/pulse irradiation were observed, cavities were cone-shaped, and deeper than those irradiated by 100mJ/pulse irradiation. There was no smear layer, and opening of odontoblasts was observed. The deepest part of the cavity showed a scaled surface (Fig.4).

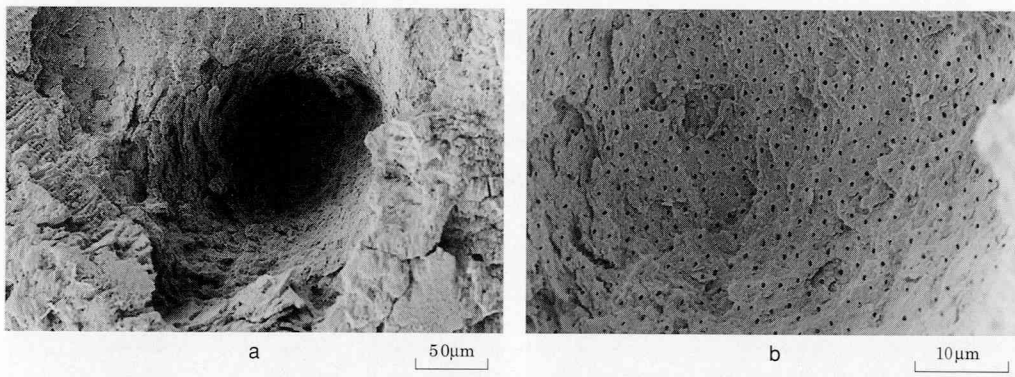


Fig.4 SEM photograph

The tooth surfaces cut with Er : YAG laser was look a conic.
a : Irradiation condition 150mJ/pulse 10pps
b : Higher magnification of Fig. 4a

Carbonization or generation of cracks was not detected under any conditions of irradiation.

2) Experiment 2 : The effects of heat generated by irradiation-in vitro-

(1) Changes in the temperature after irradiation

Under all irradiation conditions, the temperature slowly increased during laser irradiation until it reached the maximal level, and started to slowly decrease immediately after irradiation, but after some time, the temperature remained at a certain level.

When the laser was applied to the dentin of 1.0mm thickness at 50, 100 and 150mJ/pulse, the temperature increased by 1.3 ± 1.1, 2.9 ± 1.4, and 4.0 ± 1.8℃, respectively (Fig.5). When speci-

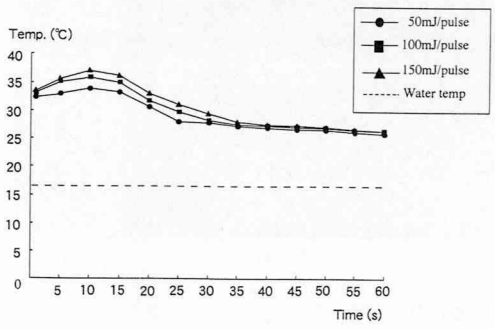


Fig.5 Thermal changes by different irradiation condition
(Thickness of dentin 1.0mm)

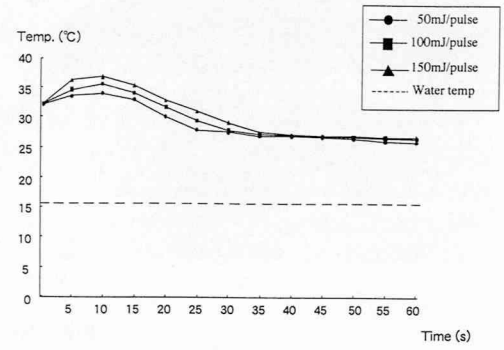


Fig.6 Thermal changes by different irradiation condition
(Thickness of dentin 1.5mm)

mens of 1.5mm thickness were irradiated at 50, 100, and 150mJ/pulse, the temperature increased by 1.1 ± 0.8, 2.3 ± 1.4, and 3.3 ± 1.8℃, respectively (Fig.6).

(2) Difference in thermal changes according to the thickness of dentin

Table 4 shows difference in the temperature according to dentin thickness for each condition of irradiation.

Under all irradiation conditions, the increase in the temperature decreased as the thickness of specimens increased, but increased as the irradiation energy increased from 50, 100 to 150 mJ/pulse.

As for 2s irradiation, as the thickness of dentin increased from 1.0mm to 1.5mm, rise in the temperature decreased from 0.5 ± 0.3 to 0.1 ± 0.1℃ for 50mJ/pulse irradiation, from 1.2 ± 1.0 to 0.9 ± 0.6℃ for 100mJ /pulse irradiation, and from 2.9 ± 1.5 to 2.3 ± 1.5℃ for 150mJ/pulse irradiation (Fig.7).

For 5s irradiation, as the thickness of dentin increased from 1.0mm to 1.5mm, an increase in the temperature decreased from 1.1 ± 0.9 to 0.8 ± 0.6℃ for 50mJ/pulse irradiation, from 2.0 ± 1.1 to 1.7 ± 0.9℃ for 100 mJ/pulse irradiation, from 3.8 ± 2.2 to 3.1 ± 1.9℃ for 150mJ/pulse irradiation (Fig.8).

For 10s irradiation, as the thickness of dentin increased from 1.0mm to 1.5mm, an increase in the temperature decreased from 1.3 ± 1.1 to 1.1 ± 0.8℃ for 50mJ/pulse irradiation, from 2.9 ± 1.4 to 2.3 ± 1.4℃ for 100 mJ/pulse irradiation, and from 4.0 ± 1.8 to 3.3 ± 1.8℃ for 150mJ/pulse irradiation (Fig.9).

(3) Difference in thermal changes due to the difference in the irradiation time

Under all irradiation conditions, the rise in the temperature increased as the irradiation time increased or as the irradiation energy increased from 50, 100 to 150mJ/pulse.

As for the dentin of 1.0mm thickness, as

Table 4 Thermal changes by different thickness of dentin

(℃)

	Irradiation time											
	2s				5s				10s			
Irradiation condition	1.0mm AVE.±S.D.		1.5mm AVE.±S.D.		1.0mm AVE.±S.D.		1.5mm AVE.±S.D.		1.0mm AVE.±S.D.		1.5mm AVE.±S.D.	
50mJ/pulse	0.5	0.3	0.1	0.1	1.1	0.9	0.8	0.6	1.3	1.1	1.1	0.8
100mJ/pulse	1.2	1.0	0.9	0.6	2.0	1.1	1.7	0.9	2.9	1.4	2.3	1.4
150mJ/pulse	2.9	1.5	2.3	1.5	3.8	2.2	3.1	1.9	4.0	1.8	3.3	1.8

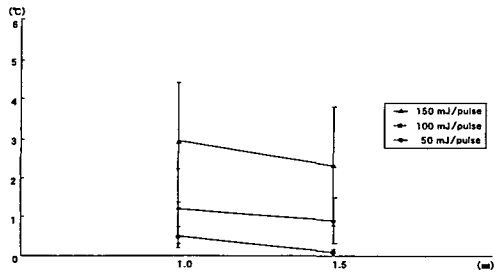


Fig.7 Thermal changes by different thickness of dentin
(Irradiation time 2s)

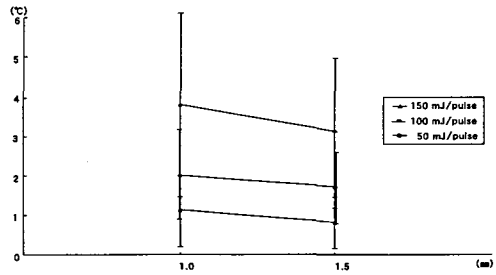


Fig.8 Thermal changes by different thickness of dentin
(Irradiation time 5s)

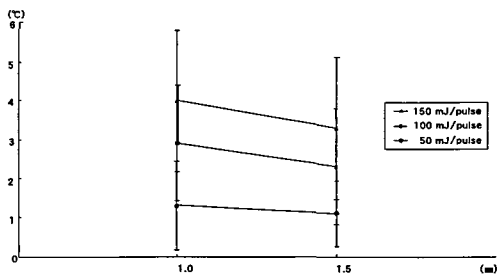


Fig.9 Thermal changes by different thickness of dentin
(Irradiation time 10s)

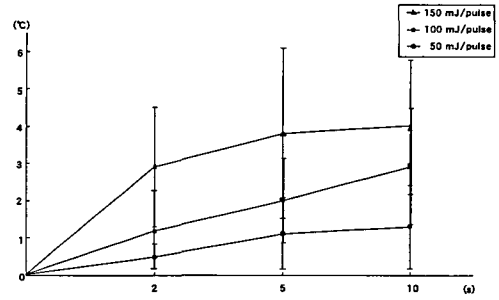


Fig.10 Thermal changes by different irradiation time
(Thickness of dentin 1.0mm)

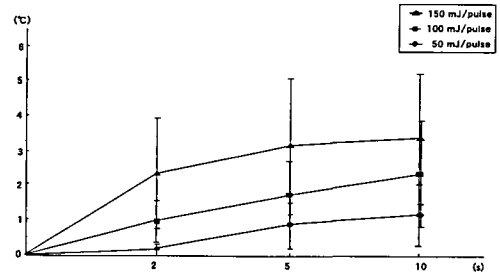


Fig.11 Thermal changes by different irradiation time
(Thickness of dentin 1.5mm)

the irradiation time increased from 2s to 10s, the increase in the temperature increased from 0.5 ± 0.3 to 1.3 ± 1.1℃ for 50mJ/pulse irradiation, from 1.2 ± 1.0 to 2.9 ± 1.4℃ for 100mJ/pulse irradiation, from 2.9 ± 1.5 to 4.0 ± 1.8℃ for 150mJ/pulse irradiation (Fig.10).

For dentin of 1.5mm thickness, as the irradiation time increased from 2s to 10s, the increase in the temperature increased from 0.1 ± 0.1 to 1.1 ± 0.8℃ for 50mJ/pulse irradiation, from 0.9 ± 0.6 to 2.3 ± 1.4℃ for 100mJ/pulse irradiation, from 2.3 ± 1.5 to 3.3 ± 1.8℃ for 150mJ/pulse irradiation (Fig.11).

3) Experiment 3 : Histopathological observation

The histopathological findings for each observation period are shown in Table 5–7.

Table 5 Histopathological findings (Immediately)

Rats (No.)	1	2	3	4	5	6	7	8	25	26	27	28	29	30	31	32	49	50	51	52	53	54	55	56
Irradiation condition (mJ/pulse)	50								100								150							
Minimum thickness of dentin (μm)	180	200	160	180	200	170	180	200	170	180	160	150	170	160	190	180	160	140	150	130	120	130	160	140
Hyperemia	±	±	+	+	−	±	±	−	±	±	±	+	±	+	±	±	±	+	+	+	++	++	+	+
Hemorrhage	−	−	−	−	−	−	−	−	±	±	±	+	−	+	±	±	±	+	+	+	++	+	+	+
Inflammatory cell infiltration	−	−	±	±	−	−	−	−	±	±	±	±	−	+	−	−	±	±	±	+	+	+	±	+
Irregularity, atrophy, disappearance, vacuolization of odontoblast layer	±	−	±	±	−	±	−	−	±	±	±	±	±	+	±	−	±	+	+	+	+	+	±	±
Disappearance of pulpo-dentinal membrane	±	−	±	±	−	±	−	−	±	±	±	±	±	+	±	−	±	±	±	+	+	+	±	±
Rod spaped body in dentinal tuble	−	−	−	−	−	−	−	−	−	±	±	±	−	±	−	−	−	−	±	±	±	±	±	−
Degeneration, necrosis of pulp cell proper	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−
Formation of osteodentin	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Proliferation of pulp cell proper	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Fibrosis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Table 6 Histopathological findings (7 days after irradiation)

Rats (No.)	9	10	11	12	13	14	15	16	33	34	35	36	37	38	39	40	57	58	59	60	61	62	63	64
Irradiation condition (mJ/pulse)	50								100								150							
Minimum thickness of dentin (μm)	210	180	160	180	200	180	180	200	180	160	160	150	180	160	150	170	130	140	160	110	150	130	120	120
Hyperemia	−	−	±	±	−	±	±	−	−	±	±	±	±	−	±	±	±	±	−	+	±	±	+	±
Hemorrhage	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	±	−	−	−	−
Inflammatory cell infiltration	−	−	±	−	−	±	−	−	−	−	±	±	−	±	±	−	−	−	−	±	−	−	±	±
Irregularity, atrophy, disappearance, vacuolization of odontoblast layer	−	−	±	±	−	±	±	−	±	±	±	±	±	±	±	−	±	±	±	+	±	±	+	±
Disappearance of pulpo–dentinal membrane	−	−	±	−	−	−	−	−	−	−	−	±	−	±	±	−	−	±	−	±	−	−	±	±
Rod spaped body in dentinal tuble	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Degeneration, necrosis of pulp cell proper	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	±	−
Formation of osteodentin	−	−	±	−	−	−	−	−	−	−	−	±	−	±	±	−	±	±	−	±	−	−	±	−
Proliferation of pulp cell proper	−	−	±	±	−	±	±	−	±	−	−	±	−	±	±	±	±	±	±	+	±	±	+	−
Fibrosis	−	−	±	−	−	−	−	−	−	−	−	±	−	±	±	−	−	±	−	±	−	−	±	−

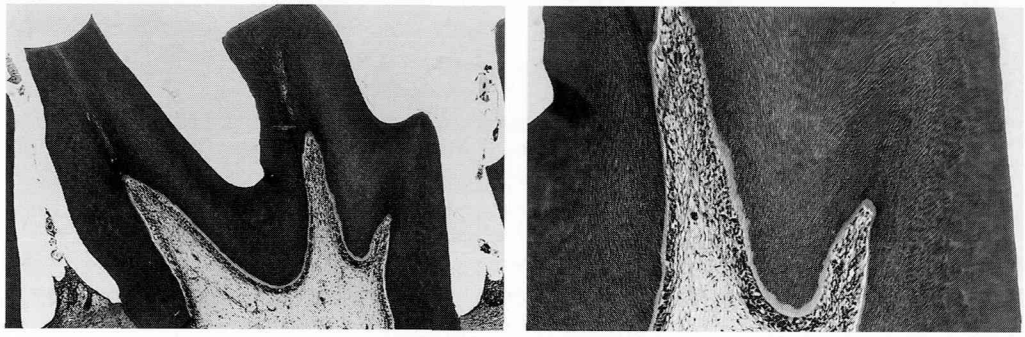
Table 7 Histopathological findings (30 days after irradiation)

Rats (No.)	17	18	19	20	21	22	23	24	41	42	43	44	45	46	47	48	65	66	67	68	69	70	71	72
Irradiation condition (mJ/pulse)	50								100								150							
Minimum thickness of dentin (μm)	180	150	180	160	200	160	150	180	160	150	180	200	160	150	180	160	140	160	120	120	150	140	120	130
Hyperemia	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Hemorrhage	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Inflammatory cell infiltration	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Irregularity, atrophy, disappearance, vacuolization of odontoblast layer	−	−	−	−	−	−	±	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	±	−
Disappearance of pulpo–dentinal membrane	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Rod spaped body in dentinal tuble	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Degeneration, necrosis of pulp cell proper	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Formation of osteodentin	−	−	−	−	−	−	−	−	±	±	−	−	±	±	−	−	±	−	±	±	±	±	±	−
Proliferation of pulp cell proper	−	±	±	±	−	±	±	−	±	±	−	−	−	−	−	−	−	−	±	±	±	±	±	−
Fibrosis	−	−	−	−	−	−	±	−	−	±	−	−	−	±	−	−	−	−	±	−	±	±	±	−

(1) Immediately after irradiation

a. Control group

Formation of only a small amount of physiological secondary dentin was observed. The pulp cavity was large with the projection of the horns, and a small amount of reparative dentin was found only around the pulp horns in some teeth. The three layer structure consisting of the odontoblastic layer, cell-free layer, and cell-rich layer was clear (Fig.12).



a ×65 b ×163

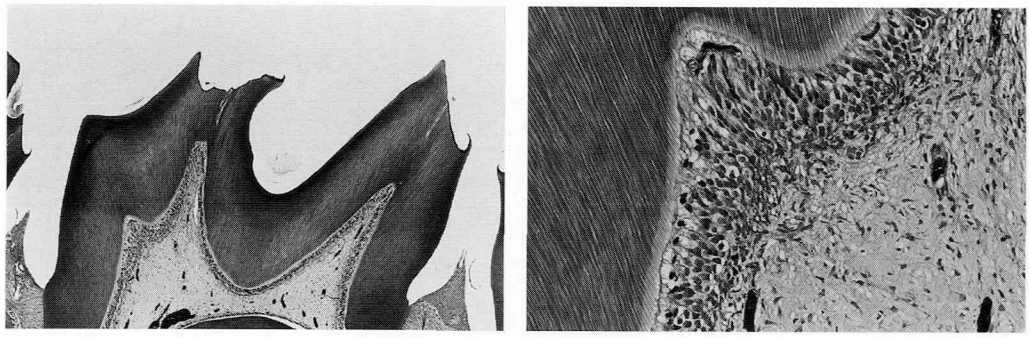
Fig.12 Control (Immediately)

a : There was only slight deposition of physiological secondary dentin, and the pulpal horns were clearly projected with a small amount of reparative dentin limited to the horns.
b : The three-layer structure, consisting of an odontoblastic layer, a cell-free layer and a cell-rich layer, was apparent.
(Higher magnification of Fig. 12a)

b. Laser cutting group

For 50mJ/pulse irradiation, the minimal thickness of dentin ranged from 160 to 200μm with the mean value of 184μm. There was slight hyperemia in the coronary pulp chamber. Slight vacuolation and intercellular edema were observed in the odontoblastic layer, and there were only a small number of round cells. Partial disappearance of the pulpo-dentinal membrane was also detected (Fig.13).

For 100mJ/pulse irradiation, the minimal thickness of dentin ranged from 150 to 190μm



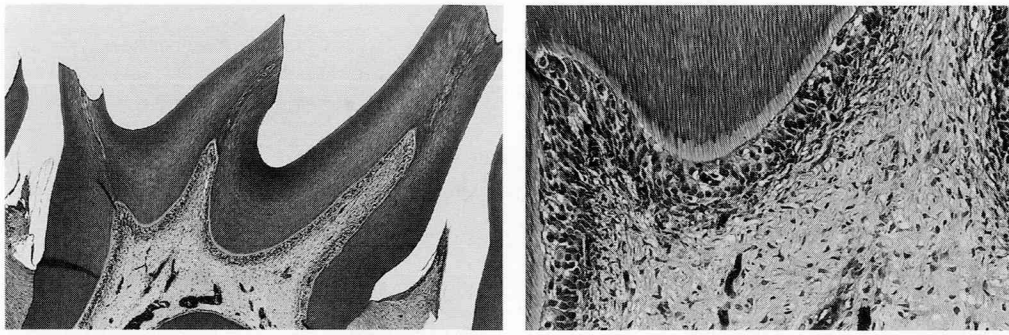
a ×65 b ×325

Fig.13 50mJ/pulse (Immediately after laser irradiation)

a : There was slight hyperemia in the coronal pulp.
b : Slight vacuolization and intercellular edema were observed in the odontoblastic layer. There were very few round cells. A partial loss of the pulpo-dentinal membrane was noted.
(Higher magnification of Fig. 13a)

with the mean value of 170μm. There was slight hyperemia in the coronary pulp chamber, and aspiration of some red cells was observed (Fig.14–1). In the odontoblastic layer, severe vacuolation and intercellular edema were found in a wide area, associated with the disappearance of the pulpo–dentinal membrane. Leaking hemorrhage was observed in a comparatively wide area in the coronary pulp chamber, but no cells exhibited coagulative necrosis (Fig.14–2).

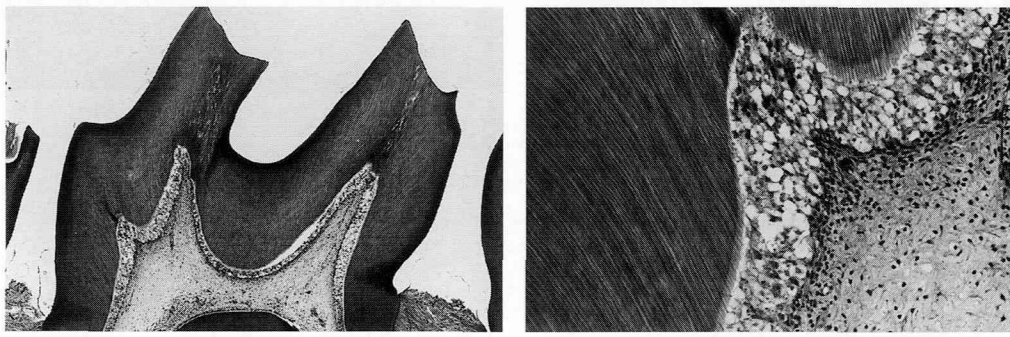
For 150mJ/pulse irradiation, the minimal thickness of dentin ranged from 120 to 160μm with the mean value of 141μm. Odontoblasts in the coronary pulp chamber exhibited vacuolation with disappearance of the pulpo–dentinal membrane. These denatured cells were underlined by extensive monocyte infiltration. Hyperemia accompanying leaking hemorrhage was noted in the entire pulp. There was also disarray in the arrangement and disappearance of odontoblasts, and even in specimens showing comparatively mild changes in the arrangement of odontoblasts, rods that were odontoblasts aspirated into dentinal tubules were observed. In one specimen with the minimal thickness of 120μm, the focus of necrosis was detected imme-



a　×65　　b　×325

Fig.14–1 100mJ/pulse (Immediately after laser irradiation)

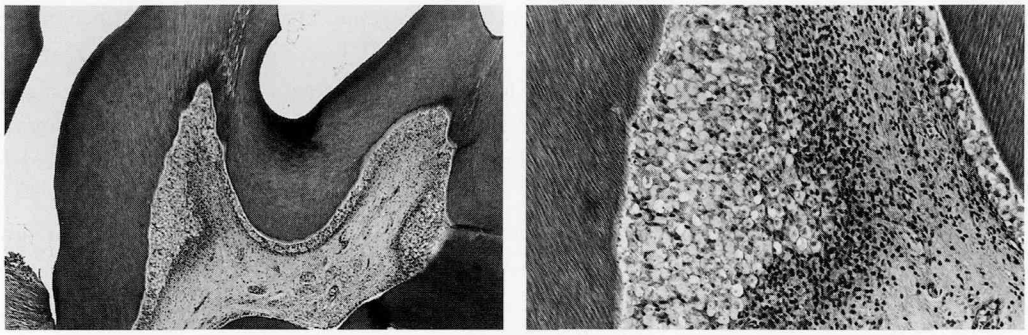
a : There was slight hyperemia in the coronal pulp.
b : Some erythrocytes were aspirated in dentinal tubules.
(Higher magnification of Fig. 14–1a)



c　×65　　d　×325

Fig.14–2 100mJ/pulse (Immediately after laser irradiation)

c : Extensive and marked vacuolization and intercellular edema were detected in the odontoblastic layer, accompanied by a loss of the pulpo–dentinal membrane.
d : Hemorrhage per diapedesin was observed in a comparatively wide area of the coronal pulp, but there was no coagulative necrosis of the cells.
(Higher magnification of Fig. 14–2c)



a ×82 b ×325

Fig.15 150mJ/pulse (Immediately after laser irradiation)

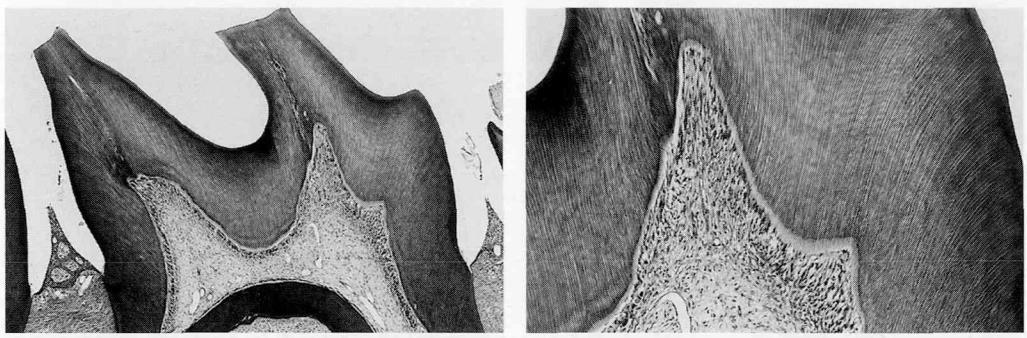
a : Odontoblasts in the coronal pulp presented vacuolization with an extensive loss of the pulpo-dentinal membrane.
b : Marked monocyte infiltration was found immediately beneath these denatured cells. Hyperemia accompanying hemorrhage per diapedesin was noted in the entire pulp. (Higher magnification of Fig. 15a)

diately under the irradiated surface (Fig.15).

(2) Seven days after irradiation

a. Control group

It was found that formation of physiological secondary dentin and reparative dentin increased with age. The three layer structure consisting of the odontoblastic layer, cell-free layer, and cell-rich layer was clear (Fig.16).



a ×65 b ×163

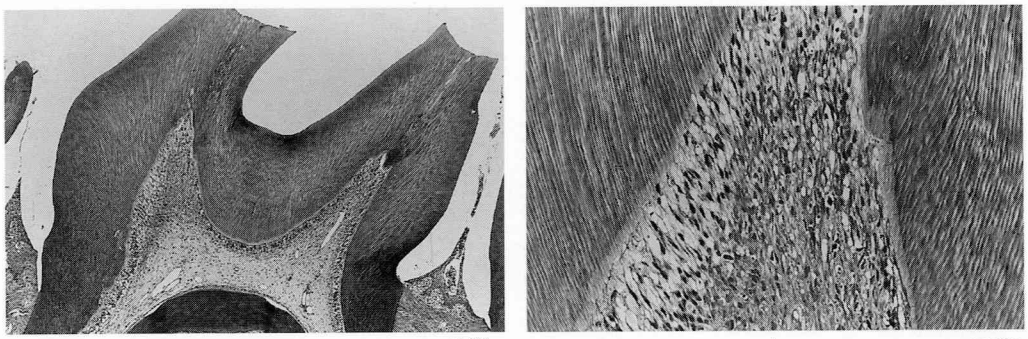
Fig.16 Control (7days after)

a : The amount of deposition of physiological secondary dentin and reparative dentin in the pulpal horns increased with age.
b : The three-layer structure of the odontoblastic layer, cell-free layer and cell-rich layer was apparent. (Higher magnification of Fig. 16a)

b. Laser irradiation group

For 50mJ/pulse irradiation, the minimal thickness of dentin ranged from 160 to 210μm with the mean value of 186μm. There was little hyperemia or exudative changes in the coronary pulp chamber. The number of odontoblasts slightly decreased, and they showed reticular changes. Hyperplasia of pulp cells proper was observed (Fig.17).

For 100mJ/pulse irradiation, the minimal thickness of dentin ranged from 150 to 180μm

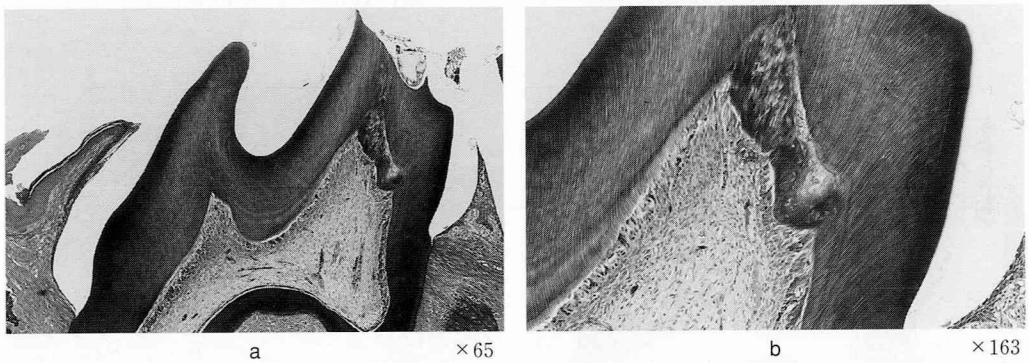


a　×65　　b　×325

Fig.17 50mJ/pulse (7days after laser irradiation)

a : There was only slight hyperemia and few exudative changes in the coronal pulp.
b : The number of odontoblasts was slightly decreased in some part, where odontoblasts undergoing reticular changes and proliferation of pulpal cells were observed.
(Higher magnification of Fig. 17a)

with the mean value of 164μm. Although hyperplasia of pulp cells proper was seen, hyperemia or changes of odontoblasts were scarce. In addition to the formation of physiological secondary dentin, formation of osteodentin containing cells was noted in the area facing the cavity (Fig.18).

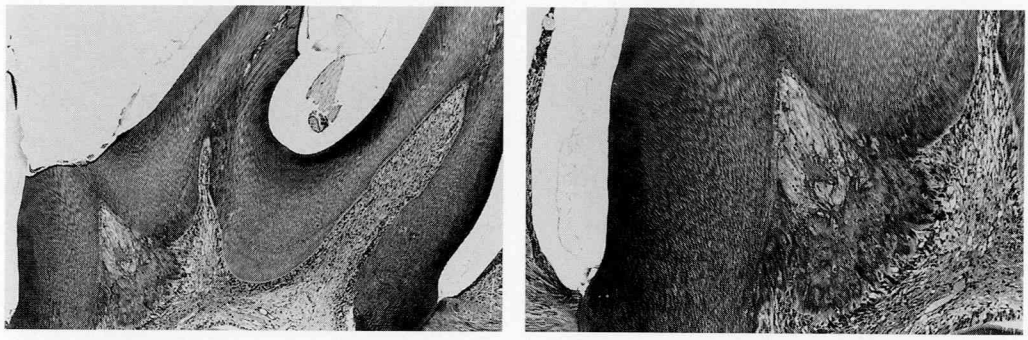


a　×65　　b　×163

Fig.18 100mJ/pulse (7days after laser irradiation)

a, b : Although proliferation of pulpal cells were observed in the coronal pulp, there was only slight hyperemia and few changes in odontoblasts.
In addition to the deposition of physiological secondary dentin, osteodentin was formed in the part corresponding to the cavity, entrapping cells.
(b : Higher magnification of Fig. 18a)

For 150mJ/pulse irradiation, the minimal thickness of dentin ranged from 110 to 160μm with the mean value of 133μm. Histological changes were similar to those for 100mJ/pulse irradiation, but a comparatively large number of osteodentin-containing cells formed in the area facing the cavity, and extensive hyperplasia of pulp cells proper was found. In two specimens with dentin thickness of 110 and 120μm, a small number of odontoblasts and pulp cells proper exhibited necrobiosis immediately under the irradiated surface. However, they also showed formation of a large amount of osteodentin and extensive hyperplasia of pulp cells proper in the same region (Fig.19).



a ×65 b ×163

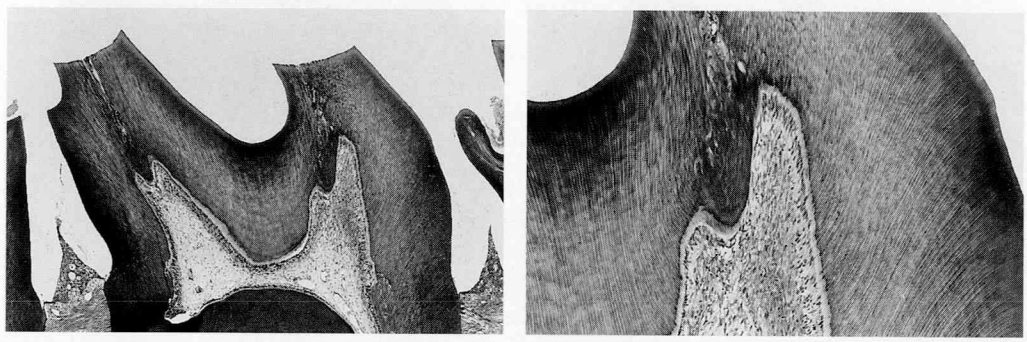
Fig.19 150mJ/pulse (7days after laser irradiation)

a, b : In the part corresponding to the cavity, a large amount of osteodentin including cells was formed with proliferation of pulpal cells in a comparatively wide area.
A small number of odontoblasts and pulpal cells undergoing degeneration and necrosis were found immediately underneath the irradiation area, where there was also formation of a large amount of osteodentin and comparatively extensive proliferation of pulpal cells.
(b : Higher magnification of Fig. 19a)

(3) Thirty days after irradiation

a. Control group

With progression of attrition, formation of secondary dentin was observed in the roof, horns, and floor of the pulp chamber, which caused a distinctive reduction in the size of the pulp chamber (Fig.20).



a ×65 b ×163

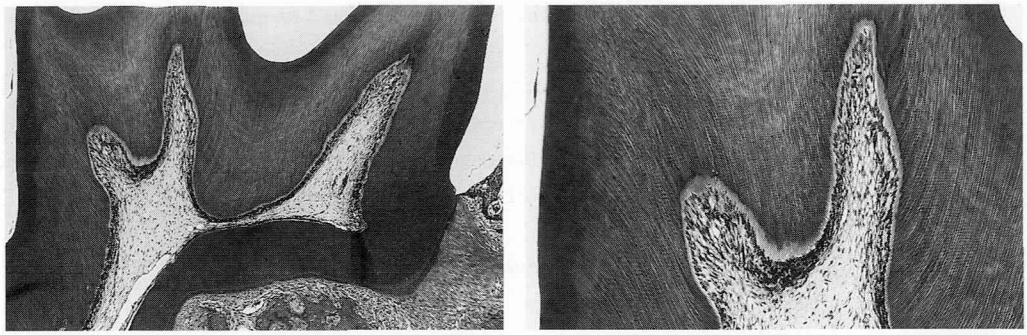
Fig.20 Control (30days after)

a, b : Because of advanced attrition , formation of secondary dentin was observed in the roof and base of the pulp chamber as well as the pulp horns, causing an apparent narrowing of the pulp chamber.
(b : Higher magnification of Fig. 20a)

b. Laser irradiation group

For 50mJ/pulse irradiation, the minimal thickness of dentin ranged from 150 to 200μm with the mean value of 170μm. With progression of attrition due to aging, extensive formation of physiological secondary dentin was observed in the roof, horns and floor of the pulp chamber, resulting in a distinctive reduction in the size of the pulp chamber. There were no other distinctive changes (Fig.21).

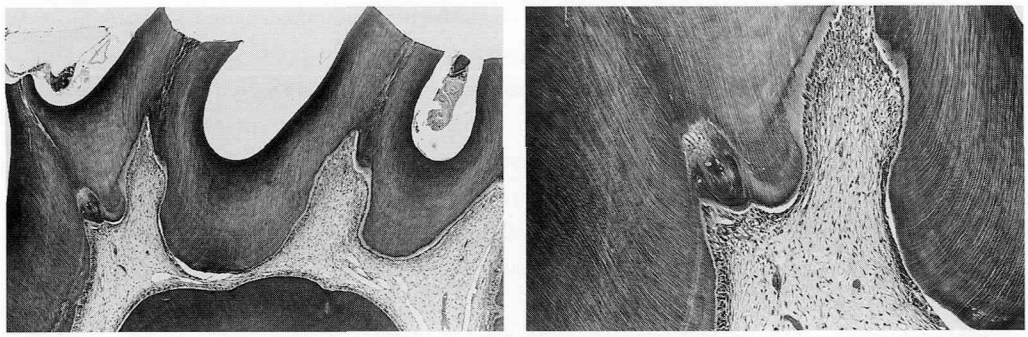
For 100mJ/pulse irradiation, the minimal thickness of dentin ranged from 150 to 200μm with the mean value of 168μm. There was neither hyperemia nor any marked changes in



a　×65　　b　×163

Fig.21 50mJ/pulse (30days after laser irradiation)

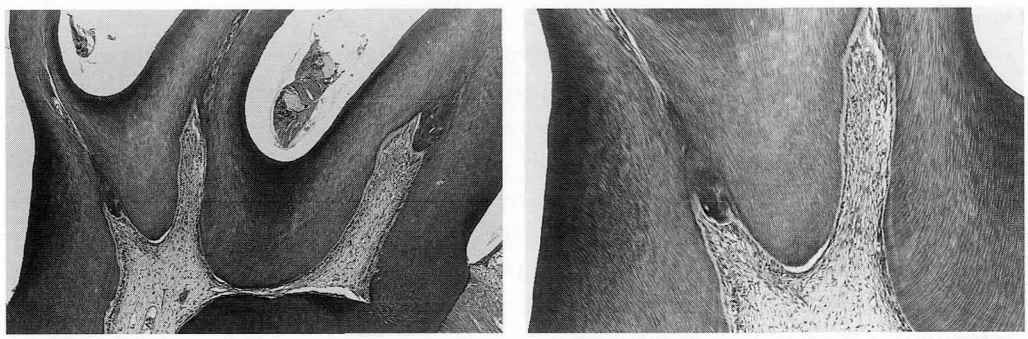
a, b : There was attrition due to aging, which resulted in a narrowed pulp chamber with extensive formation of physiologic secondary dentin in the roof and base of the pulp chamber as well as the pulp horns. No other marked changes were noted.
(b : Higher magnification of Fig. 21a)



a　×65　　b　×163

Fig.22 100mJ/pulse (30days after laser irradiation)

a, b : There was no hyperemia or marked changes in odontoblasts.
Formation of secondary dentin was observed.
Osteodentin which contains cells was also found in the part corresponding to the cavity.
(b : Higher magnification of Fig. 22a)



a　×65　　b　×163

Fig.23 150mJ/pulse (30days after laser irradiation)

a, b : There was no edema, hyperemia, or hemorrhage in odontoblasts.
There was no marked changes in the odontoblastic layer.
(b : Higher magnification of Fig. 23a)

odontoblasts. Formation of secondary dentin was observed, and formation of osteodentin that contained cells was also found in the area facing the cavity (Fig.22).

For 150mJ/pulse irradiation, the minimal thickness of dentin ranged from 120 to 160μm with the mean value of 135μm. Unlike specimens immediately and 7 days after irradiation, edema, hyperemia or hemorrhage of odontoblasts was not observed, and there were no marked changes in the odontoblastic layer (Fig.23).

Discussion

1. The animals used in the experiment

There are a number of studies using animals in observing the reactions of the pulp to laser irradiation[8,9–19]. The animals used in the experiments are mainly dogs[8,9–15], and rats[16–19]. In selecting experimental animals, it is necessary to consider whether the animal exhibits the oral conditions that are suitable for the study. In some previous reports investigating pulpal reactions to laser irradiation[9,13], teeth were cut with an air turbine handpiece to expose dentin before irradiation, which indicates that the effects of cutting with an air turbine handpiece may be mistaken for the effects of laser irradiation. Since this study aimed to perform histopathological investigation of dentin after laser irradiation, we selected murine molars, the cusps of which are not covered with enamel, so that the direct effects of laser irradiation on the pulp could be investigated.

During the experiment, the general conditions of the animals were followed by periodically measuring weight and dietary intake. There were no significant changes in the general conditions of the animals except that body weight increased due to normal physical development, and it was concluded that laser irradiation did not affect the general conditions of the animals.

2. Conditions of irradiation

Since the effects of laser irradiation are influenced by the wave length, output, pulse number of the laser as well as irradiation time, irradiation conditions should vary depending on the objective of the study. Ishimaru et al.[20] demonstrated in the study where an Er : YAG laser with a wave length of 2.94μm was used to cut teeth that cutting was possible when a laser with irradiation energy of 50, 100, and 150mJ was applied to bovine teeth, and that cutting capacity increased as the pulse number increased. Takano[21] reported that when human extracted teeth were irradiated by a laser with energy of 100, 150, or 100mJ for 10secs at 1pps or for 2secs at 5pps while spraying water on the tooth, cutting was possible, but generation of cracks was observed in the surfaces irradiated with 200mJ laser. Thus, based on these reports, we chose irradiation conditions so as to optimize the cutting capacity as well as to avoid generation of cracks.

In addition, the specimens used in this study were immature permanent teeth, which are characterized by increased possibility of the pulp being exposed to external stimuli since they have thinner dentin than mature permanent teeth. Thus, irradiation energy needs to be limited to low levels while maximized to maintain optimal cutting capacity within the permissible range. In the preliminary experiment, we first experimented with the use of a 31mJ/pulse laser to cut teeth, but cutting was impossible. Then, we gradually increased the irradiation energy until cutting became possible at 50mJ/pulse, which was established as the minimal irradiation energy.

When the laser was used at more than 150mJ/pulse, there were some specimens showing pulp exposure. Therefore, 150mJ/pulse was thought to be the maximal level. In addition, the pulse number was set at 10pps in consideration of the cutting efficiency.

3. SEM observation

We[1] performed SEM observation of the irradiated surfaces after applying a CO_2 laser, Nd : YAG laser and Er : YAG laser on dentin, and found that the outer layer of the irradiated surfaces melted and there was blockage of dentinal tubules after irradiation with the CO_2 laser and Nd : YAG laser. However, after irradiation with Er : YAG laser, there was no blockage of dentinal tubules, which is similar to the findings of the present experiment.

The blockage of tubules found after irradiation with the CO_2 laser and Nd : YAG laser is attributable to heat[22]. However, since the Er : YAG laser has a wave length of 2.94μm, which is similar to the absorption spectrum of water, it has the properties of high absorbability to water. When teeth are irradiated, laser energy converges in water surrounding hydroxyapatite crystals, which increases the internal pressure, causing explosion, and teeth destruction[3]. Since the state of dentinal tubules reflects the mechanism of cutting, it was speculated that melting and blockage of dentinal tubules of the irradiated surface are caused by the heat from the Nd : YAG laser and CO_2 laser while a flaky appearance and opening of dentinal tubules on the cut surfaces are caused by the destruction of apatite crystals rather than melting by the Er : YAG laser.

When dentin is cut with an air turbine handpiece or an engine, a smear layer is normally produced, cutting debris enter the end of tubules, and a dentinal plug is formed. However, such findings were not observed in the dentin irradiated by the Er : YAG laser, which was thought to be attributable to the difference in the mechanism of cutting.

There have been various studies concerning the smear layer. Some studies found that if the smear layer is not removed before filling, the growth of bacteria is facilitated since bacteria can propagate within the smear layer, and the fit of restorations is decreased, thus reducing the retention[23,24]. However, it was also reported that since the permeability of dentin is reduced by the presence of a smear layer and a dentinal plug in dentinal tubules, external stimuli can be blocked, preventing exudation of dentinal fluid. Thus, dry conditions inside the cavity can be maintained, preventing the physical properties and retention of the restoration from decreasing[25-28]. At present, many researchers support the former mechanism, and removing the smear layer after cavity preparation is generally accepted[29-31]. The Er : YAG laser does not produce a smear layer unlike the other two lasers, indicating that it is useful for cavity preparation

4. The effects of heat

Even if laser cutting of immature permanent teeth is feasible, clinical application cannot be realized unless the effects of the laser on the pulp of immature permanent teeth are investigated. Therefore, we performed in vitro experiments using human immature permanent teeth prior to the histopathological examination. To create a condition resembling the actual oral condition at the time of laser irradiation in association with clinical application, the specimens were placed on a sponge soaked in artificial saliva, which was maintained at 37℃, before irradiation. Since the aim of this study was to investigate the effects of heat on the pulp when the thickness of remaining dentin after laser irradiation was 1.0 and 1.5mm rather than to irradiate dentin, the contact tip was fixed during irradiation.

Water supplied during irradiation greatly influences the effects of heat[32,33]. Takizawa[15] reported that water affected the changes in the temperature and the morphology of the irradiated surfaces, and that the temperature rose about 7℃ after irradiation while supplying water. Also in the present study, water was supplied during irradiation in consideration of the influence of heat. The amount of water supply was set at 25ml/min in the present study since carbonization and cracks

were detected in some parts of the cut surfaces after irradiation using a high energy laser even when water was supplied at 20ml/min. However, further studies should be done to determine whether 25ml/min of water is appropriate. Although no carbonization or cracks were observed under any conditions by SEM, and elevation of the temperature was limited to less than 5℃ in most rats during the final experiment, some rats showed more than a 5℃ increase in the temperature, indicating that an increase in water supply could have prevented temperatures from rising more than 5℃ in all animals. However, Bhasker et al. noted that excessive cooling can become a harmful stimulus to the pulp[32], and that excessive water supply can reduce the cutting efficiency[33]. Thus, we concluded that the amount of water supply should be discussed by considering its cooling effects as well as the cutting efficiency of the laser.

In measuring changes in the temperature following laser irradiation, thermocouples[16,34-36] and thermograms[37-39] are used. It is known that when the laserbeam is absorbed directly by the thermocouple, the heat generated by the laser can be included in the measurement. However, it was also reported[40] that the use of the needle elements of a thermocouple is suitable for measuring changes in the temperature caused by laser irradiation. Prior to the final experiment, we examined whether a thermocouple actually measures only the heat absorbed into the tissue. After irradiating the thermocouple under the same conditions as in the final experiment, changes in the temperature were recorded. It was found that the results differed from those obtained in the final experiment, where direct laser irradiation was performed on the specimens. The temperature increased rapidly on direct laser irradiation, and decreased rapidly on completion of irradiation, after which the temperature remained at a constant level.

In the final experiment, however, the temperature started to increase slowly with a slight time lag after initiation of laser irradiation, and started to decrease slowly immediately after completion of irradiation, after which the temperature remained at a constant level.

These findings suggested that the changes in the temperature recorded by a thermocouple indicate the heat absorbed into the tissue. Furthermore, when specimens of various thicknesses were irradiated under various conditions, there was no perforation of the specimens toward the thermocouple caused by cutting, and when the changes in the temperature were compared between 50 mJ/pulse irradiation to the specimen with a thickness of 1.5mm and 150mJ/pulse irradiation to the specimen with a thickness of 1.0mm, the temperature increased and decreased slowly as described above. Therefore, there is little possibility of direct irradiation on the thermocouple.

In a study examining the effects of heat on the pulp, Zach and Cohen[41] demonstrated necrosis of the pulp in 15% of the vital teeth when the increase in the temperature of the pulpal wall exceeded 5.6℃ for 5 to 20s in an experiment using monkeys. It is widely accepted that more than a 5 ℃increase in the temperature affects the pulpal tissues[42,43]. Sugata et al[42]. found in an experiment using a CO_2 laser that when irradiation was performed in the SP mode with an output of 1W (mean output : 0.15W), irradiation time of 3-10s, and energy density of 117-390J/cm^2 on dentin of more than 1.5mm thickness, the increase in the temperature of the pulp was less than 5℃. We also reported that when a CO_2 laser was applied in the SP mode with an output of 2W (mean output : 0.2-0.6W), irradiation time of 2-10s, and energy density of 68-204J/cm^2, the increase in the temperature was less than 5℃ if dentin was more than 1.0mm thick[44]. Takizawa[15] demonstrated that when an Er : YAG laser was irradiated at 200mJ/pulse for 5s, at 100mJ/pulse for 60s, and at 200 mJ/pulse for 60s, the temperature increased by 6.7℃, less than 6.5℃ and less than 7.1℃, respectively. According to a report by Machida et al[37]., the temperature did not increase even after

250 mJ/pulse irradiation. In the present study, the temperature rose less than 5℃ under all conditions of irradiation with any irradiation time for all specimens, indicating that heat has little influence on the pulp when laser irradiation is performed with water supply.

Hamilton et al.[45] and Matsumura et al[46]. demonstrated that the effects of heat and other elements on the pulp tissues are closely related to the thickness of dentin. Since immature permanent teeth, which have just erupted, are characterized by the projection of the pulpal horns and the large chamber, it was necessary to clarify the relationship between dentin thickness and the effects of heat on the pulp tissues. It was found that although it was difficult to compare lasers with different wave lengths because of their properties, the Er : YAG laser influences the pulp tissues less than the CO_2 laser even when dentin is only 0.5mm thick. This suggests the usefulness of laser application to immature permanent teeth.

5. Histopathological examination

Since the effects of heat on the pulp are closely associated with the thickness of dentin[45,46], it was necessary to measure the thickness of dentin after laser irradiation in rats. As specimens were prepared by demineralization, dehydration and paraffin embedding, the obtained values should be slightly larger than actual values because of slight shrinkage of the specimens during the process of specimen preparation.

The association between the remaining dentin after cavity preparation and reaction of the pulp has previously been studied[47,48]. It was reported that strong inflammation was observed when dentin was less than 1,000 μm thick in human teeth[47]. It was also reported that some reactions occurred when dentin was less than 400μm thick[48]. Wang[11] found that when a Nd : YAG laser was applied to dentin in dogs, hemorrhage was observed in a wide area of the pulp and there was also vacuolation and necrosis of the pulp tissues when the thickness of dentin was less than 350μm. It was also found in the same experiment that inflammation remained 28 days after irradiation, resulting in delayed healing. Sekine[12] reported that after irradiation with an Er : YAG laser, there was hemorrhage in pulp tissues, the appearance of rods within dentinal tubules, and necrobiosis of pulp tissues when dentin was less than 500μm thick. It was also found that 28 days after irradiation, inflammation was still observed, and healing was delayed. Thus, reactions of the pulp increased as the thickness of dentin decreased. Takizawa[15] noted the inflammation 90 days after irradiation when the dentin thickness was less than 500μm. In the present study, we examined the relationship between dentin thickness and changes in temperature in vitro, and found that the pulp is influenced by heat in some specimens when the thinnest part of dentin is 500μm thick. When these reports and our in vitro data are considered, the pulp seems prone to inflammation as dentin was quite thin with thicknesses of 110 to 210μm after laser irradiation in the present study. However, there was no severe inflammation in the pulp except when dentin thickness was less than 120μm (immediately after 150mJ/pulse irradiation in 1 specimen, and 7 days after 150 mJ/pulse irradiation in 2 specimens). In these cases, necrobiosis of the pulp was observed only beneath the irradiated surface. In addition, there were three specimens having dentin with a thickness of less than 120μm that were observed for 30 days after 150mJ/pulse irradiation, and necrobiosis was not detected in pulp tissues in any of these specimens. It was speculated that the actual increases in the temperature were less than those expected partly because the heat was dispersed through blood flow[16]. However, since the effects of heat caused by irradiation depends on cooling by water supply, irradiation energy, and dentin thickness, water supply is thought to have decreased the histological changes in pulp tissues by limiting the rise in the temperature to about

less than 5℃ as already described in the discussion of the effects of heat. In cases where significant changes in the temperature were observed, it is possible that the temperature increased more than 5℃.

Next, the relationship between the condition of irradiation and reactions of the pulp is discussed. Although we used an Er : YAG laser in the experiment, as did Sekine[12] and Takizawa[15], our findings differed from theirs, which may be attributed to the difference in the conditions of irradiation. The highest energy of the laserbeam was 200mJ/pulse[12], and 250mJ/pulse[15] in their studies while the highest energy of the laserbeam was 150mJ/pulse in the present study. Thus, irradiation energy was lowed in our study. There were few changes in the histopathological findings immediately after irradiation with 50mJ/pulse laser, and when irradiation energy was increased to 100mJ/pulse and 150mJ/pulse, inflammation extended from immediately under the irradiated surface to a wide area of the pulp. Hemorrhage, inflammatory cell infiltration, disarray in the arrangement, disappearance and vacuolation of odontoblasts, disappearance of the pulpo-dentinal membrane and aspiration of the nuclei of odontoblasts as well as erythrocytes increased. However, with time, these inflammatory changes recovered under all conditions of irradiation. Therefore, although cutting of a tooth causes a certain degree of damage to the pulp immediately after irradiation, these inflammatory changes seem reversible. These findings suggest that all the conditions of irradiation used in the present study can be used in cutting immature permanent teeth, and that 50mJ/pulse irradiation caused little damage while cutting was feasible. In addition, a focus of necrosis was not observed after 100mJ/pulse irradiation. Therefore, it was concluded that the optimal conditions of irradiation are within the limits of 50 and 100mJ/pulse. Although these conditions may not be immediately applied to human teeth, this study confirmed the usefulness and safety of the application of the Er : YAG laser to immature permanent teeth.

Regarding the relationship between the condition of irradiation and remaining dentin, the cutting capacity increases as the irradiation energy increases. In this study, the mean thickness of dentin after irradiation was 180, 167 and 136μm for 50, 100 and 150mJ/pulse irradiation, showing that the thickness was least for 150mJ/pulse irradiation. The thinner the remaining dentin becomes, the larger the effects of thermal changes and physical stimuli are, thus increasing histological changes. Although animals that received 150mJ/pulse irradiation showed the largest changes in this study, the precise relationship between the irradiation energy and histological changes caused by irradiation can not be discussed unless the thickness of dentin after irradiation is unified.

It has previously been reported that the Nd : YAG laser and CO_2 laser facilitated the formation of secondary dentin[11,13], but as for the Er : YAG laser, it was found that the formation of secondary dentin was increased only when the remaining dentin was thin[15], and the increase was slight[12]. This study showed similar findings as formation of osteodentin was observed when the remaining dentin was comparatively thin. The formation of osteodentin was also observed where odontoblasts were thought to have undergone degeneration or disappeared. Such osteodentin is considered to be generated by neoodontoblasts that originate from undifferentiated mesenchymal cells, which proliferate and migrated from the cell-rich layer, where odontoblasts had disappeared, or odontoblasts differentiated from pulp cells proper. When the Er : YAG laser was used to cut dentin, the heat and the pressure produced by pulse irradiation influence the pulp[15]. If dentin is thin, these stimuli can be transmitted to the pulp, increasing the formation of osteodentin. In this study, histological observation revealed that 30 days after irradiation, odontoblasts and pulp cells

proper, which underwent degeneration caused by excessive stimuli, recovered their function, and even after necrosis, cells were regenerated, and dentin matrix and fibrous matrix were formed by odontoblasts and pulp cells proper, respectively. As the cusps of murine molars lack enamel, there is active formation of reparative dentin due to attrition with age and physiological secondary dentin. Therefore, it is not necessarily easy to distinguish whether the formation of secondary dentin is physiologic or results from attrition or laser irradiation. In this study, additional dentin formed beneath the cavity that had a decreased number of dentinal tubules as well as inclusion cells regarded as osteodentin, which was considered the secondary dentin resulting from irradiation. However, as reparative dentin does not always show findings of osteodentin, there might have been little formation of reparative dentin in animals which received 50mJ/pulse irradiation when observed 30 days after irradiation.

Follow-up was performed until 30 days after irradiation in the present study. Immediately after irradiation, acute inflammatory reactions such as circulatory disturbance, i. e. hyperemia and hemorrhage, in the pulp, disarray in the arrangement of odontoblasts and disappearance of the pulpo-dentinal membrane as well as vacuolation were observed. Seven days after irradiation, formation of osteodentin and proliferation of pulp cells proper were detected. Thirty days after irradiation, there were no significant changes compared to the control group. Thus, the reactions of the pulp to irradiation recovered almost completely. However, some rats showed degeneration even 30 days after irradiation, indicating the necessity of continuing observation more than 30 days after irradiation.

Conclusion

This study investigated the usefulness and safety of Er : YAG laser application to cavity preparation of immature permanent teeth. In the experiment, murine upper incisors, and lower first and second molars that are in the middle of development as well as human immature permanent premolars with incomplete apices were irradiated by applying an Er : YAG laser, and SEM observation of the irradiation surface, investigation of the effect of heat caused by irradiation, and histopathological observation were performed, and the following results were obtained.

1. SEM observation of the irradiation surface revealed that under all conditions of irradiation, there was no formation of smear layer nor dentinal plug generally after cutting with an air turbine handpiece, but opening of dentinal tubules was observed.

   Since no carbonization or cracks were observed under any condition of irradiation, cavity preparation of immature teeth may be physically feasible, and cutting with the Er : YAG laser was different from that with high-speed instruments.

2. When the effects of heat were examined, it was found that the increase in the temperature caused by irradiation was affected by the irradiation energy, irradiation time and thickness of dentin, and as the irradiation energy increased, the irradiation time increased, or the thickness of dentin increased, and the increase in the temperature increased.

   Furthermore, the temperature of dentin close to the pulp rose after laser irradiation, but owing to sufficient water supply, the mean rise in the temperature was less than 5℃ under all conditions of irradiation, indicating that irradiation is not harmful to the pulp.

3. Histopathological observation revealed circulatory changes such as hyperemia, hemorrhage and exudative changes, disarray in the arrangement of odontoblasts, and disappearance of the pulpo-

dentinal membrane immediately after irradiation. It was also found that as the irradiation energy increased, histological changes in the pulp increased. However, recovery was observed 7 days after irradiation under all conditions of irradiation, when formation of osteodentin and propagation of pulp cells proper were detected. These significant changes were not observed 30 days after irradiation, showing that the effects on the pulp were reversible.

Thus, it was indicated that cutting of immature permanent teeth using an Er : YAG laser may be feasible. When its effects on the pulp observed immediately after irradiation are considered, we found that 50–100mJ/pulse irradiation is safer and more useful for immature permanent teeth.

This paper has been appeared in two reports of the Japanese Journal of Pediatric Dentistry (37 : 775–781, 782–797, 1999). These papers were concentrated to the present paper.

References

1) Kawabata A, Kawabata H, Kondo Y, Miyazawa H, Akahane S, Kamiya M and Takei K (1998) The effects of laser irradiation on dentin–First report : Morphological study of changes in dentin caused by in vitro application of different types of lasers–. Matsumoto Shigaku **24** : 115–23 (in Japanese)
2) Kumazaki M, Fujiwara H, Matsuda T, Zennyu K, Toyoda K and Fujii B (1992) Excision of dental caries. J Jpn Soc Laser Dent **3** : 23–7
3) Kumazaki M and Toyoda K (1995) Removal of hard dental tissue (Cavity preparation) with the Er : YAG laser. J Jpn Soc Laser Dent **6** : 14–6
4) Hibst R and Keller U (1993) The mechanism of YAG laser induced ablation of dental hard substances. S P I E **1880** : 156–62
5) Tsuchiya T and Kurosu K (1996) A study of child Galvanic Skin Reflex (GSR) reaction to various dental stimuli–Important psychological factors groverning GSR wave patterns–. J J Psy Dent **11** : 59–67. (in Japanese)
6) Ohhashi T (1986) Pulpal response of human dental pulp to Glass ionomer cements (Fuji Ionomer Type I and II). Jpn J Conserv Dent **29** : 33–68. (in Japanese)
7) Shimada Y (1992) Pulpal responses various dentin conditioning agents. Jpn J Conserv Dent **35** : 69–100. (in Japanese)
8) Fujimori F (1976) Microvascular alteration in the dental pulp induced by the cavity preparation with ruby laser and engine. Odontology **63** : 756–70. (in Japanese)
9) Shouji S , Nakamura M , Horiuchi H and Yamamoto H (1982) The changes of human dentin and dental pulp by Nd : YAG laser irradiation. J Jpn Soc Laser Med **3** : 599–604. (in Japanese)
10) Kimura K, Shinoki K, Yamada K, Momono H, Kamei T, Huruuchi Y, Chiba J, Fujita Y, Hayashi S and Yamamoto H (1984) Experimental study of the effects of CO_2 laser radiation on tooth and dental pulp. J Jpn Soc Laser Med **4** : 267–8.
11) Wang H (1990) Histopathological changes of dental pulp after Nd : YAG laser irradiation to dog tooth cavity. Jpn J Conserv Dent **33** : 1643–58. (in Japanese)
12) Sekine Y (1995) Histopathological study of Er : YAG laser application to cavity preparation. Jpn J Conserv Dent **38** : 211–33. (in Japanese)
13) Takizawa M, Seino E, Aoki S, Takase Y, Hirai Y, Takahashi K and Ishikawa T (1996) Histopathological study of pulp response to CO_2 laser irradiation in adult dogs. Jpn J Conserv Dent **39** :

24–37. (in Japanese)
14) Kumazaki M and Kumazaki M (1996) Histopathological study of tooth by Er : YAG laser application to dental hard tissue. J J Dent Mater **15** : 104–15. (in Japanese)
15) Takizawa M (1996) A study on Er : YAG laser ablation for dental hard substances. Jpn J Conserv Dent **39** : 1089–128. (in Japanese)
16) Matsui K (1981) Laser irradiation and temperatures of dental pulp in vital teeth. Reports Inst Med Dent Eng **15** : 129–33. (in Japanese)
17) Nagasawa A, Yamamoto H, Yoshino T, Kato K and Ohshiro T (1985) Fundmental studies in reactive histological changes in pulp tissues of lased teeth. J Jpn Soc Laser Med **5** : 413–6. (in Japanese)
18) Nakamura Y (1987) Histopathological changes of dental pulp of rats after irradiation by Nd : YAG laser. J Stomatol Soc Jpn **54** : 705–21. (in Japanese)
19) Kato J, Hashimoto M and Ono H (1989) Pulp reactions of the rat developing molars after pulpotomy with Nd : YAG laser irradiation. J Jpn Soc Laser Med **10** : 521–4. (in Japanese)
20) Ishimaru K, Takano T, Asano S, Kusaka T, Chen C, Sakakibara Y, Takamizu M, Gotoh S and Kohno A (1996) The influence of Erbium : YAG laser on the tooth surface morphology. Jpn J Conserv Dent **39** : 1229–39. (in Japanese)
21) Takano T (1997) A study on morphological and component change of tooth surface irradiated by Er : YAG laser Part 1. SEM observation and EDS analysis of lased cavity floor. Jpn J Conserv Dent **40** : 908–19. (in Japanese)
22) Matsumoto K (1993) A guide to using lasers in dentistry. 116–20. Quintessence, Tokyo. (in Japanese)
23) Brannstrom M (1984) Smear layer pathological and treatment consideration. Operative Dent Supplement **3** : 35–42.
24) Dahl B L (1978) Effect of cleaning procedures on the retentive ability of two luting cements to ground dentin in vitro. Acta Odont Scand **36** : 137–42.
25) Vojinivic O, Nyborg H and Brannstrom M (1973) Acid treatment of cavities under resin fillings : Bacterial growth in dentinal tubules and pulpal reactions. J Dent Res **52** : 1189–93.
26) Reeder Jr O W, Walton R E, Livingston M J and Pashley D H (1978) Dentin permeability, Determinations of hydraulic conductance. J Dent Res **57** : 187–93.
27) Michelich V, Pashley D H and Whitford G M (1978) Dentin permeability : a comparison of functional versus anatomical tubular radii. J Dent Res **57** : 1019–24.
28) Pashley D H, Michelich V and Kehl T (1981) Dentin permeability, Effect of smear layer removal. J Prosthet Dent **46** : 531–7.
29) Hosoda H (1988) Smear layer removal with pretreatment agents. The Journal of Dental Medicine **28** : 392–400. (in Japanese)
30) Hasegawa K and Katoh Y (1990) Study on the effects of various cavity cleansing agents on the dentine wall in the cavity–Comparison of cleansing effects by using SEM and image analyzer–. Jpn J Conserv Dent **33** : 1319–52.
31) Hosoya Y, Tominaga A and Goto G (1994) Resin adhesion to the primary dentin. Report 2. Jpn J Ped Dent **32** : 785–800. (in Japanese)
32) Bhasker S N and Lilly G E (1965) Intrapulpal temperature during cavity preparation. J Dent Res **44** : 644–7.
33) Burkes E J, Hoke J, Gomes E and Wolbarsht M (1992) Wet versus dry enamel ablation by Er :

YAG laser. J Prosthet Dent **67** : 847–51.
34) Yamada K (1985) Study on cariess prevention by laser irradiation in pediatric dentistry'. Jpn J Ped Dent **23** : 575–91. (in Japanese)
35) Senda A, Gomi A, Hara G, Miyajima K, Iizuka T, Hasegawa J, Sugata F and Yuuzu T (1993) Thermal changes of dentin by CO_2 laser irradiation–Thermal transmissions in lateral and vertical directions–. Aichi–Gakuin Dent Sci **6** : 25–31.
36) Kato K, Nagasawa A, Nishikawa K, Hiratsuka Y, Kawahara T, Atsumi K (1980) Basic study on change of temperature of a tooth after CO_2 laser irradiation. J Jpn Soc Laser Med **1** : 294–300. (in Japanese)
37) Machida T, Mazeki K, Narushima K and Matsumoto K (1996) Study on temperature raising in tooth structure at irradiating Er : YAG laser. J Jpn Endo Assoc **17** : 38–40. (in Japanese)
38) Kumazaki M (1996) Removal of hard dental tissue (cavity preparation) with the Er : YAG laser. J Jpn Soc Laser Med **17** : 1–8.
39) Matsumoto K, Choukan S, Hibi H, Fukuyama K, Nakamura Y and Shirasuga T (1990) Study on application of pulsed Nd : YAG laser in concervative dentistry. J Showa Univ Dent Soc **10** : 41–5. (in Japanese)
40) Nagasawa A, Kato K and Nishikawa K (1982) Studies of true temperature measurments with the improved thermocouple. J Jpn Soc Laser Med **3** : 195–200. (in Japanese)
41) Zach L and Cohen G (1965) Pulp response to externally applied heat. Oral Surg **19** : 515–30.
42) Sugata F, Ohmori K, Tanaka T, Yuuzu T, Senda A, Gomi A, Kamiyama K, Hara G and Hasegawa J (1994) The morphological study of dentin irradiation by CO_2 laser (The 2nd report) –Influence of thermal increase–. Jpn J Conserv Dent **37** : 384–9. (in Japanese)
43) Gomi A, Irinoda M, Matsui O, Kamiya K, Senda A, Hara G, Hasegawa J and Sugata F (1994) Morphological, chemical and physical changes of dentin irradiate by CO_2 laser. Aichi–Gakuin Dent Sci **32** : 229–36. (in Japanese)
44) Kawabata A, Kawabata H, Yagasaki A, Iwasaki H and Miyazawa H (1999) The effects of heat a carbon dioxide laser on dentin–A comparison of normalpulse laser and superpulse laser–. Jpn J Ped Dent **36** : 823–30. (in Japanese)
45) Hamilton B G, Robinson L and William L (1962) Operative dentistry and the pulp. J Pros Dent **12** : 985–1001.
46) Matsumura Y, Kato M, Hotta D and Kurosu K (1979) Influence of a new adhesive fillling material (Clearfil bond system–F). Jpn J Ped Dent **17** : 427–33. (in Japanese)
47) Harold R S (1976) Human pulp response to operative dental procedures, 46–52. Storter Printing Co., Florida.
48) Mitchell D F, Buonocore M G and Shazer S (1962) Pulp reaction to silicate cement and other materials, Relation to cavity depth. J Dent Res **41** : 591–5.

抄録：幼若歯への Er : YAG レーザー切削の応用

川端明美，岩崎　浩，矢ヶ崎アンジェリ，宮沢裕夫（松本歯大・小児歯科）

Er : YAG レーザーを幼若歯の窩洞形成に応用した場合の有用性および安全性を検索する目的で成長発育期にあるラットの歯牙および根尖未完成なヒト幼若小臼歯を用いて Er : YAG レーザーによる歯質

の切削を行い，適切な照射条件の設定，切削面のSEM観察，照射による熱影響，病理組織学的観察を行った．その結果，切削面のSEM観察ではエアータービンによる切削時にみられるsmear layerやdentinal plugは観察されず，象牙細管は開口していた．さらに，50，100，150mJ/pulseの照射条件で炭化やクラックの発生は認められなかった．また，熱影響の検討では象牙質の厚径1.0，1.5㎜で照射時間2，5，10秒で50，100，150mJ/pulseのそれぞれの組み合わせによる照射条件下での歯髄側象牙質の平均温度は5℃未満であった．病理組織学的観察では照射直後では循環障害や象牙芽細胞の配列の乱れが観察されたが，1週後では回復傾向を示す所見が認められた．

以上のことからEr:YAGレーザーによる歯質切削は可能であるが歯髄への影響を考慮すると，幼若歯の歯質切削時は50～100mJ/pulseでの照射が有用かつ安全と考えられた．