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Author(s)	Aizawa, K; Kameyama, A; Kato, J; Oda, Y; Hirai, Y
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## Nano-Hardness of Adhesive Interface between Er:YAG Laser-Irradiated Dentin and 4-META/MMA-TBB Resin

KOYA AIZAWA, D.D.S.,<sup>1</sup> ATSUSHI KAMEYAMA, D.D.S., Ph.D.,<sup>1</sup> JUNJI KATO, D.D.S., Ph.D.,<sup>1</sup>  
YUTAKA ODA, B.Sc., Ph.D.,<sup>2</sup> and YOSHITO HIRAI, D.D.S., Ph.D.<sup>1</sup>

### ABSTRACT

**Objective:** The purpose of this study was to evaluate the hardness of the adhesive interface between resin and Er:YAG laser-irradiated bovine dentin by nano-indentation. **Background Data:** It has been reported that laser output energy and pulse repetition rate affect the tensile bond strength in Er:YAG laser-irradiated dentin. **Materials and Methods:** Three laser settings were evaluated at the same total energy level (approximately 1.0 W): 100 mJ/pulse-10 pps (100-10), 50 mJ/pulse-20 pps (50-20), and 33 mJ/pulse-30 pps (33-30). Laser-irradiated dentin in each group was conditioned with 10% citric acid solution containing 3% ferric chloride for 15 sec, rinsed with distilled water for 30 sec, and bonded to PMMA rods with 4-META/MMA-TBB resin. The bonded specimens were sectioned vertically, embedded in epoxy resin, and their nano-hardness measured. A non-irradiated control group was also investigated. **Results:** The adhesive resin interface in the controls showed the lowest level of hardness, which gradually increased from the top of the hybrid layer (0  $\mu\text{m}$ ) through the bottom of the hybrid layer (5  $\mu\text{m}$ ) and into the underlying dentin ( $\sim 10$   $\mu\text{m}$ ). Significant differences in hardness were observed between the 5  $\mu\text{m}$  point in the controls, the 10  $\mu\text{m}$  and 15  $\mu\text{m}$  points in the 100-10 group, and the 10  $\mu\text{m}$  point in the 50-20 and 33-30 groups. **Conclusion:** The results suggest that laser settings affect hybrid layer thickness, even when the total energy level is constant.

### INTRODUCTION

MOST STUDIES OF RESIN BONDING to Er:YAG laser-irradiated dentin or marginal adaptation to Er:YAG laser-prepared cavities have reported poorer results than those seen with non-irradiated dentin/cavities.<sup>1,2</sup> One suggested reason for this is the denaturing of the organic components of dentin due to heat generation, despite cooling with a water spray.<sup>3-6</sup> Furthermore, laser-irradiated surfaces show a unique morphology, including the orifices of dentin tubules, no smear layers, and flaky and irregular intertubular dentin,<sup>1</sup> constituting changes in mechanical properties that may compromise bond strength.

Increases in output energy or pulse frequency have also been reported to affect bond strength,<sup>7,8</sup> possibly due to changes in the mechanical properties of the laser-irradiated surface. Furthermore, in an earlier study, we evaluated the relationship between output energy/pulse frequency and tensile bond strength

at the same total energy level, and found that a lower output energy/higher pulse frequency decreased bond strength, possibly due to differences in the mechanical properties of the laser-modified dentin.<sup>9</sup>

Investigations of the hardness of the resin-dentin adhesive interface<sup>10-13</sup> have almost exclusively employed nano-indentation,<sup>10-12</sup> a technique effective for determining the hardness of the submicron hybrid layer on a scale much smaller than that possible with micro-hardness testing. As the subsurface of Er:YAG-laser-irradiated dentin morphologically changed at a depth of 60–190  $\mu\text{m}$ ,<sup>5,14</sup> this technique may be more effective in analyzing the adhesive interface between resin and Er:YAG-laser-irradiated dentin.

The purpose of this study was to evaluate the influence of three different combinations of output energy and pulse frequency at the same total laser energy level on the hardness of the adhesive interface using the nano-indentation technique.

<sup>1</sup>Department of Operative Dentistry, and <sup>2</sup> Department of Dental Materials Science, Tokyo Dental College, Chiba, Japan.

TABLE 1. TEST GROUPS AND LASER PARAMETERS

Group	Output energy	Repetition rate	Total energy
100-10	100 mJ/pulse	10 pps	1.0 W
50-20	50 mJ/pulse	20 pps	1.0 W
33-30	33 mJ/pulse	30 pps	1.0 W
Control	Non-irradiated		0 W

## MATERIALS AND METHODS

### Laser device and group protocol

This study used a prototype Er:YAG laser device (J. Morita Mfg. Corp., Kyoto, Japan) set to emit a wavelength of 2.94  $\mu\text{m}$ . The output energy of this laser can be adjusted within a range of 30–250 mJ/pulse, and pulse frequency can be adjusted within a range of 1–30 pulses per second (pps). The total energy delivered by the end of the probe, however, can only reach about 1.2 W. Specimen teeth were divided into three groups, each irradiated at a different output energy and pulse frequency, and a non-irradiated control group (Table 1). The total energy of each laser-irradiated group was approximately 1.0 W. A 600- $\mu\text{m}$  diameter straight-type contact probe was used. Energy levels were measured periodically with a power meter (Lasermate-P; Coherent Co., Santa Clara, CA, USA).

### Specimen preparation

Specimen preparation followed the protocol of our previous investigation on bond strength.<sup>9</sup> Twelve extracted bovine in-

cisors, frozen to maintain freshness, were defrosted and cut at the cervix immediately before specimen preparation. The coronal sides of the cut surfaces were sequentially abraded under a stream of water with SiC paper (180-, 400-, and 600-grit) to prepare flat dentin surfaces. They were randomly divided into four groups consisting of three teeth each. Nine teeth were then irradiated uniformly with the Er:YAG laser under a fine water spray on an X-Y movable stage (D212; Suruga Seiki, Shizuoka, Japan) set to travel at a scanning speed of 1.0 mm/sec.

The dentin surfaces in each group were then conditioned with 10 wt% citric acid solution containing 3 wt% ferric chloride (Green Activator; Sun Medical, Moriyama, Japan) for 15 sec, rinsed with distilled water for 30 sec, and then dried. The conditioned surfaces were bonded to square PMMA rods (8.0  $\times$  8.0  $\times$  8.0 mm) using 4-META/MMA-TBB resin (Super-Bond C&B, Sun Medical) by the brush dip method. The bonded specimens were kept at room temperature for 60 min immediately after preparation, and then stored for 24 h in water maintained at 37°C. The bonded teeth were then serially sectioned vertically using a low-speed diamond saw (Isomet<sup>TM</sup>; Buehler, Lake Bluff, IL, USA).

The sectioned surfaces were embedded in epoxy resin (Scandiplex SCAN-DIA; H.P. Tempelmann GmbH & Co., Hagen, Germany). After 1 d, the sections of adhesive interface were sequentially ground with 1200-grit SiC paper and finished with 0.05- $\mu\text{m}$  diameter alumina to obtain a mirror-like polished surface.

### Nano-hardness measurements

The adhesive interface of each specimen was measured for nano-hardness with a nano-indentation hardness measuring de-

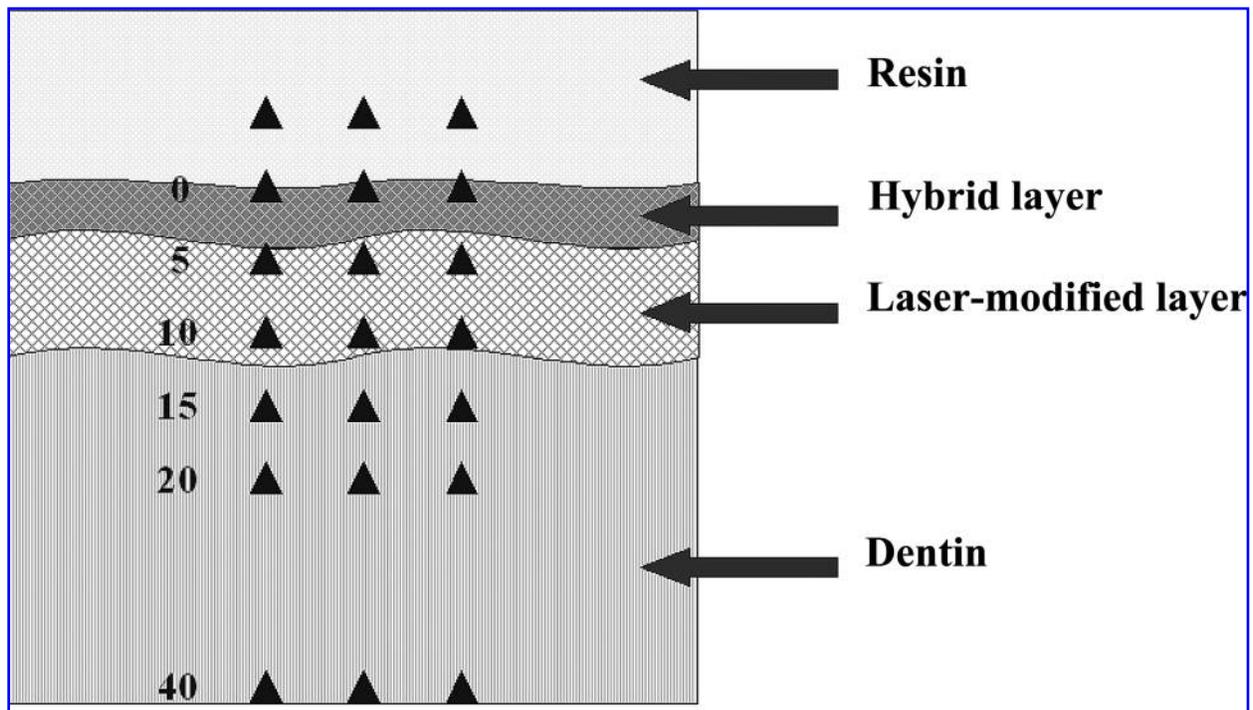


FIG. 1. Measurement points for nano-hardness testing.

TABLE 2. NANO-HARDNESS VALUES IN EACH GROUP (MEAN ± SD; MGF/μm<sup>2</sup>; N = 9)

	100-10	50-20	33-30	Control
Cured resin	39.7 ± 2.7 <sup>aA</sup>	39.7 ± 3.8 <sup>aA</sup>	39.0 ± 2.8 <sup>aA</sup>	38.7 ± 2.5 <sup>aA</sup>
0 μm	48.1 ± 5.1 <sup>aA</sup>	49.9 ± 5.9 <sup>aA</sup>	46.2 ± 3.8 <sup>aA</sup>	48.4 ± 6.3 <sup>aA</sup>
5 μm	72.8 ± 8.2 <sup>bA</sup>	76.4 ± 8.6 <sup>bA</sup>	70.1 ± 14.7 <sup>bA</sup>	102.5 ± 16.3 <sup>bB</sup>
10 μm	74.5 ± 8.6 <sup>bA</sup>	103.9 ± 13.7 <sup>cB</sup>	102.6 ± 8.8 <sup>cB</sup>	116.3 ± 8.8 <sup>cC</sup>
15 μm	106.1 ± 17.2 <sup>cA</sup>	116.1 ± 9.6 <sup>cA</sup>	110.1 ± 8.1 <sup>cA</sup>	121.4 ± 12.9 <sup>cB</sup>
20 μm	112.1 ± 14.3 <sup>cA</sup>	114.6 ± 10.0 <sup>cA</sup>	112.0 ± 7.3 <sup>cA</sup>	120.4 ± 17.2 <sup>cA</sup>
40 μm	108.5 ± 19.7 <sup>cA</sup>	115.1 ± 12.1 <sup>cA</sup>	105.5 ± 11.0 <sup>cA</sup>	111.7 ± 11.1 <sup>cA</sup>

Mean values designated by the same small letter and capital letter were not significantly different for same group/same depth from top of hybrid layer (*p* > 0.05).

vice (ENT-1100a; Elionix, Tokyo, Japan). Measurements were taken at intervals starting from a point 40 μm away from the adhesive interface at 0 μm (Fig. 1). Measuring conditions were as follows: the measuring interval was set at 5 μm vertically from the interface and 10 μm horizontally along the interface, making a total of three measuring lines per specimen; test loading was 100 mgf; and the loading/unloading rate was 0.01 mgf/msec. The data obtained from a total of nine measuring lines (three indented lines × three specimens) were recorded at the points set in the cured resin area, the top of the hybrid layer (0 μm), and at 5, 10, 15, 20, and 40 μm, and statistically analyzed with one-way and two-way ANOVA and Fisher's protected least significant difference test at a 95% level of confidence using StatView 5.0J software (SAS Institute, Cary, NC, USA).

SEM observation of adhesive interface

The surface of the specimens was etched with 6 mol/L hydrochloric acid for 30 sec, deproteinized with 1% sodium hypochlorite for 10 min, and then rinsed with water. The specimens were dehydrated in ascending grades of ethanol, dried in a desiccator for at least 1 d, gold-sputter coated with the Super Fine Coater (ESC-101; Elionix), and examined by scanning electron microscopy (SEM) (ERA-8900FE; Elionix).

RESULTS

Nano-hardness measurements

The results of the nano-hardness measurements are shown in Table 2 and Fig. 2. The ANOVA revealed a significant dif-

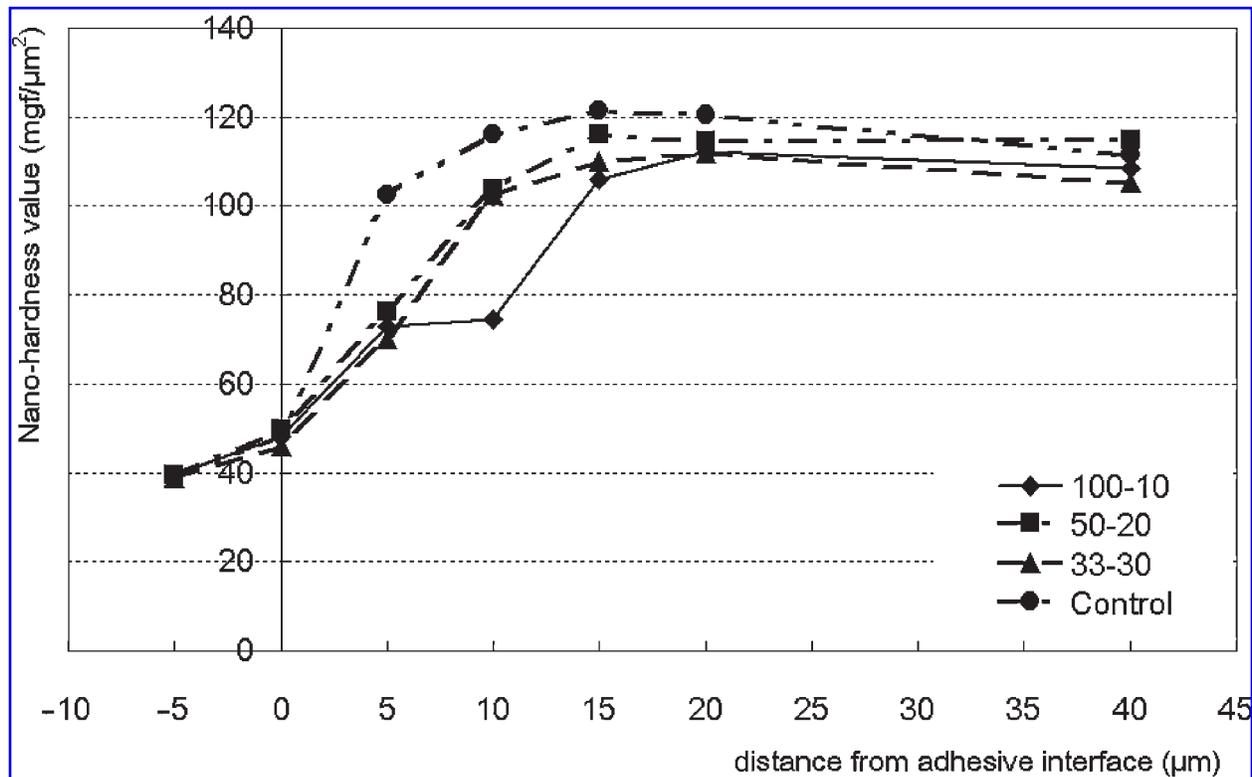


FIG. 2. Mean nano-hardness values for each group.

ference for both “depth from adhesive interface” and “laser setting,” at  $p < 0.05$ . There were no significant differences among the four groups in the cured resin area ( $p > 0.05$ ). Hardness at the top of the hybrid layer (0- $\mu\text{m}$  point) was significantly higher than that in the cured resin area in each group ( $p < 0.05$ ), and no significant differences were found among the four groups ( $p > 0.05$ ). In the non-irradiated control group, hardness at the 5- $\mu\text{m}$  point showed a sharp increase, and was significantly higher than that in the three laser-irradiated groups ( $p < 0.05$ ). Hardness showed a slight increase, but there were no significant differences among the 10-, 15-, and 20- $\mu\text{m}$  points ( $p > 0.05$ ).

In the 100-10 group, hardness showed a slight increase up to the 20- $\mu\text{m}$  point, but no significant differences were found between the 5- and 10- $\mu\text{m}$  points or the 15- and 20- $\mu\text{m}$  points ( $p > 0.05$ ). Hardness at the 10- $\mu\text{m}$  point was significantly lower in the 100-10 group than in the 50-20 and 33-30 groups ( $p < 0.05$ ). There were no significant differences between the 50-20 and 33-30 groups at any measurement point ( $p > 0.05$ ).

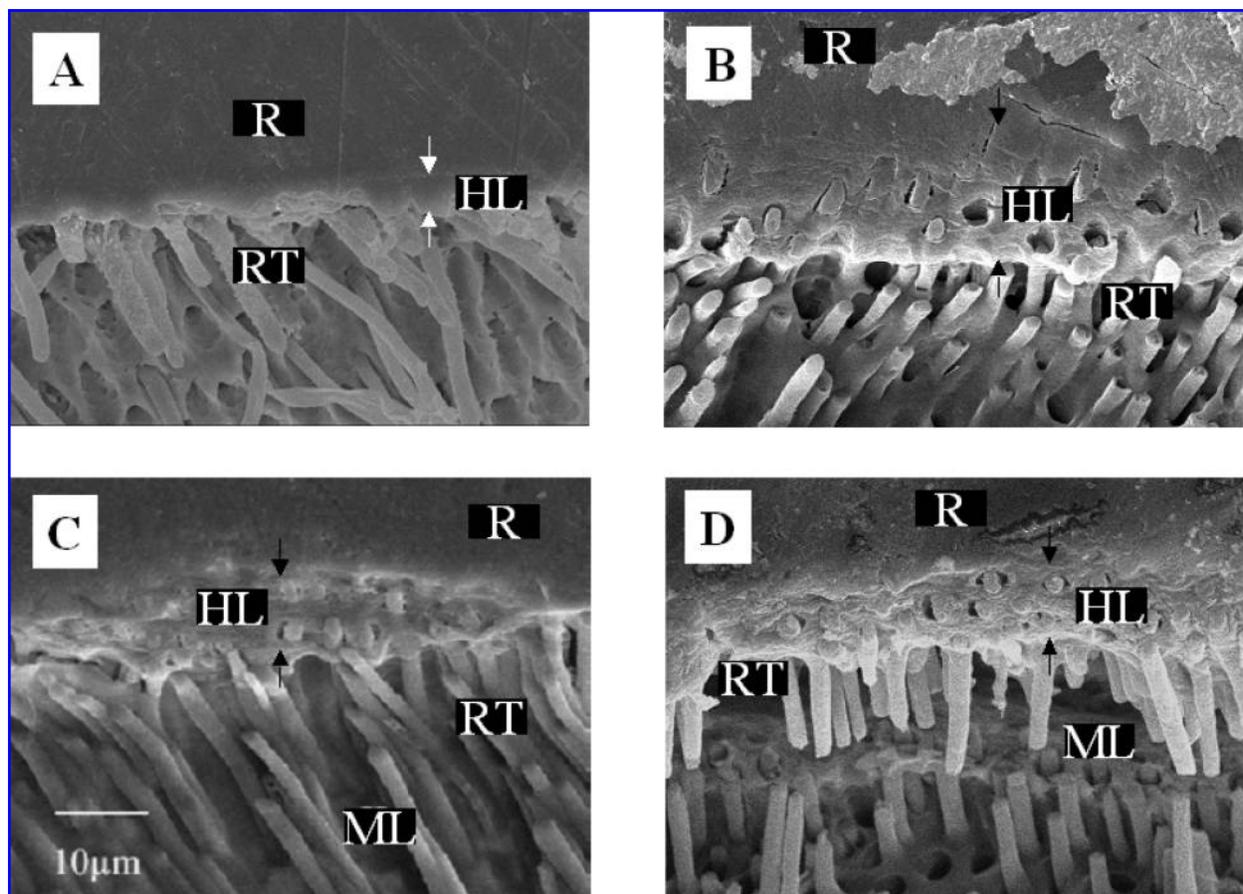
### SEM observation of adhesive interface

In the non-irradiated controls, the interface between the cured resin and underlying dentin consisted of three layers: a cured resin layer, a hybrid layer about 3–5  $\mu\text{m}$  thick, and an underlying intact dentin layer with resin tags. No gaps were observed at the resin-dentin interfaces.

The interface between the cured resin and the laser-irradiated dentin in the 100-10 group was composed of four layers: resin, a hybrid layer, a laser-modified layer, and an underlying dentin layer. The hybrid layer and the irregular layer had a thickness of about 5  $\mu\text{m}$  and 15  $\mu\text{m}$ , respectively, and both were clearly visible. Resin tags extended into the laser-modified layer.

## DISCUSSION

No significant differences were observed in nano-hardness among the four groups tested at either cured resin or 0  $\mu\text{m}$ .



**FIG. 3.** SEM micrographs of adhesive interface. (A) Sample from the non-irradiated control group, showing the 3- to 5- $\mu\text{m}$  thick hybrid layer (HL) and resin tags (RT). (B) Sample from the 100-10 group. Note the thicker hybrid layer (HL) with resin tags (RT). (C) Sample from the 50-20 group, and (D) the 33-30 group. The hybrid layer is approximately 10  $\mu\text{m}$  thick. Resin tags (RT) are also visible. ML, laser-modified dentin layer.

However, at 5  $\mu\text{m}$ , nano-hardness in the controls was significantly higher than that in the three laser-irradiated groups. SEM of the controls revealed that the thickness of the hybrid layer was about 5  $\mu\text{m}$  (Fig. 3A), with a mean  $102.5 \pm 16.3 \text{ mgf}/\mu\text{m}^2$  as the nano-hardness at the bottom of this layer. On the other hand, in the three laser-irradiated groups, the hybrid layers were thicker than in the controls (Fig. 3B, C, and D), with 5  $\mu\text{m}$  indicated as the area of hybridization between the cured resin and the laser-irradiated/demineralized dentin.

In both the 50-20 and 33-30 groups, nano-hardness at 10  $\mu\text{m}$  was higher than that at 5  $\mu\text{m}$ , although not significantly higher than that at 5  $\mu\text{m}$  in the controls. Therefore, these values indicate the nano-hardness of the bottom of hybrid layer, and correspond to the thickness of the hybrid layer observed on SEM.

However, in the 100-10 group, no significant difference was found between 5  $\mu\text{m}$  and 10  $\mu\text{m}$ . The values at these points indicated the hybrid layer. Nano-hardness at 15  $\mu\text{m}$  was close to that at 10  $\mu\text{m}$  in both the 50-20 and 33-30 groups, and that at 5  $\mu\text{m}$  in the controls. This indicated a thicker hybrid layer than in the other groups, and SEM supported this (Fig. 3B).

In an earlier study, we found that tensile bond strength in the 100-10 group was significantly higher than that in either the 50-20 or 33-30 group. Although a thicker hybrid layer does not necessarily result in higher bond strength, deeper resin penetration into the laser-affected dentin may reinforce mechanical adhesion between resin and dentin, thereby contributing to an increase in bond strength. This may be due to the low viscosity of 4-META/MMA-TBB resin,<sup>14</sup> resulting in monomer diffusivity.

No significant differences were found among the four groups at 20  $\mu\text{m}$  or 40  $\mu\text{m}$  nor was any significant difference found between 20  $\mu\text{m}$  and 40  $\mu\text{m}$ . No resin infiltration was observed in this area, and SEM revealed a dentin substrate. Transmission electron microscopic (TEM) analyses by Ceballos et al. demonstrated that laser-irradiated dentin surfaces had a thick laser-modified dentin layer with no collagen fibrils.<sup>15</sup> They found that Single Bond adhesive (3M ESPE) penetrated only the superficial aspect of the laser-modified dentin, and partial collagen fibril denaturation was still present, even when the superficial portion of the laser-modified layer was removed by phosphoric acid etching.<sup>15</sup> Ishizaka et al. also found color variation at a maximum depth of 600  $\mu\text{m}$  in a study using polarized light microscopy, and suggested that this area represents dentin that was morphologically altered by laser irradiation.<sup>5</sup> In this study, we found that laser irradiation caused no modification of the dentin substrate.

## CONCLUSION

The results of this study suggest that laser settings affect hybrid layer thickness, even when the total energy level is constant. Further study is needed to clarify the influence of laser irradiation on nano-hardness, and the relationship between bond strength and nano-hardness.

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Address reprint requests to:  
 Dr. Atsushi Kameyama, D.D.S., Ph.D.  
 Department of Operative Dentistry  
 Tokyo Dental College  
 1-2-2 Masago, Mihama-ku  
 Chiba 261-8502, Japan

E-mail: kameyama@tdc.ac.jp

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