Development of a Novel Optimal Resin-bonding System for Er:YAG Laser-irradiated Dentin: Effects of 3-step Resin-modified Glass-ionomer Bonding System Incorporating Acid-conditioning and Priming on Initial Bond Strength to Er:YAG Laser-irradiated Dentin and its Durability after Thermal Loading

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Abstract: With the ultimate aim of developing a novel optimal dentin-bonding system for Er:YAG laser-irradiated dentin, we investigated the adhesion-promoting effects of acid-conditioning and priming with hydrophilic monomers on the bonding performance of the resin-modified glass-ionomer bonding system (RMGI) to laser-irradiated dentin. Results showed that acid-conditioning with a solution of 10% citric acid and 2% ferric chloride followed by priming with 4-methacyloxyethyl trimellitic acid and 2-hydroxyethyl methacrylate was effective for improving the early bond strength and bonding durability of RMGI to Er:YAG laser-irradiated dentin.

Key words: Er:YAG laser, Initial dentin bonding, Bonding durability, Priming, Resin-modified glass-ionomer bonding system

Introduction

When dental hard tissue, especially dentin, is irradiated with an Er:YAG laser at a power output low enough to avoid clinical pain, a denatured layer is formed that prevents commercial resin-bonding systems from forming stable bonds. Several approaches for removing the denatured layer have been developed, all of which require additional processes and are consequently time-consuming and complicated. Thus, there is a need for a bonding system that is effective and does not require removal of the denatured layer.

We previously examined the use of resin-modified glass-ionomer (RMGI) for Er:YAG laser-irradiated dentin, and found that RMGI formed more stable, albeit weaker, bonds compared with conventional resin. Furthermore, we found that priming with hydrophilic monomers such as 4-methacryloxyethyl trimellitic acid (4-MET) and 2-hydroxyethyl methacrylate (HEMA), after acid-conditioning of irradiated dentin with a solution of 10% citric acid and 2% ferric chloride promoted polymerization of hydrophilic monomers in RMGI and a primer solution, reinforced the collagen fibers within the denatured layer, and consequently reinforced the denatured layer itself, thereby improving the strength of the initial bond to the irradiated dentin.

Although this 3-step RMGI bonding system improved initial bond performance, the denatured layer remains in the dentin, and thus, bond durability needs to be examined. Therefore, we examined the effects of the 3-step RMGI bonding system, including acid-conditioning and priming, on the durability of bonds to Er:YAG laser-irradiated dentin after application of thermal loading (i.e., thermal cycling).

Materials and Methods

Laser devices

An Er:YAG laser (wavelength, 2.94 µm; Erwin AdvErl EVO; J. Morita Corp., Osaka, Japan) and a handpiece with a contact tip (diameter, 600 µm; C600F; Morita) were used.

Preparation of dentin for laser irradiation

Fifteen freshly extracted bovine incisors were used. Two cuts perpendicular to the tooth axis were made, one approximately 1.5 mm from the incisal edge and the other 2.5 mm from the root apex, using a low-speed precision saw (IsoMet; Buehler Ltd., Lake Bluff, IL). The labial side of the crown was polished using an automated grinder (EcoMet; Buehler) to expose the flat dentin surface (approximately 6.5 mm × 6.0 mm). The surface was polished using waterproof silicon carbide paper (up to 600 grit) under irrigation and then subjected to laser irradiation.

Irradiation of dentin

Three laser irradiation conditions were used: no irradiation (denoted by “0” in group names); low-output irradiation at 50 mJ/1 pps (energy density, 7.3 J/cm²; denoted by “50” in group names); and low-output irradiation at 50 mJ/1 pps followed by finishing irradiation at 150 mJ/1 pps (energy density, 7.3 J/cm² plus 20.8 J/cm²; denoted by “50+150” in group names).

Specimens were placed on an x-y movable stage, and the laser handpiece was fixed so that the tip was perpendicular to and softly touching the dentin surface. Irradiation was performed under irrigation, and the stage was moved 0.6 mm during low-output irradiation and 0.8 mm during finishing irradiation, to ensure even irradiation.

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Materials used

The materials (e.g., surface treatment agents, bonding systems, and a composite resin) used in this study are shown in Table 1. In addition, a halogen light-curing unit (XL-3000; 3M ESPE, St. Paul, MN) was used.

Dentin surface treatment and resin filling

A silicone mold (inner diameter: 6.0 mm, height: 2.0 mm) was fixed using double-sided tape on the flat dentin surface treated under one of the three irradiation conditions. The dentin surface was treated with Fuji Lute Conditioner (GC Corp., Tokyo, Japan) according to the manufacturer’s instruction. Then, Fuji Lining Bond LC (RMGI; GC) was applied to make a layer with a thickness of 20–30 μm, and irradiated for 20 s (the Fe group). An additional priming step was performed on some samples after acid-conditioning (the Pr group). More precisely, the dentin surface was treated with Self Conditioner (GC) for 10 s, and this layer was dried by gently-blowing air until the fluid component became static. Then, Clearfil AP-X resin composite (Kuraray Noritake Dental Inc., Tokyo, Japan) was placed, and irradiated for 20 s while compressing the surface of the filled resin composite using a clear plastic strip (Epitex, GC). The silicone molds were removed, and the samples were either soaked in distilled water at 37°C for 24 h (the 24-h group), or subjected to additional thermal loading (10,000 thermal cycles [TC] of 5°C for 30 s and 55°C for 30 s; the TC group). In addition, control specimens were prepared that did not receive any thermal treatment (the NT group). The experimental procedures and the specimen groups are shown in Table 2.

Test specimen preparation and microtensile bond strength test

The procedure for preparing specimens for the microtensile bond strength test is shown in Fig. 1. The samples were sectioned perpendicular to the tooth axis using the IsoMet low-speed precision saw to obtain slices of 1.0 mm thick. The slices were trimmed to obtain square pillar-shaped specimens with a resin–dentin interface area of 1.0 mm² (1.0 mm × 1.0 mm); 12 specimens were prepared for each subgroup. The ends of the test specimen were fixed on the bond strength tester (EZ Test; Shimadzu Corp., Kyoto, Japan) using a cyanoacrylate adhesive (Zapit; Dental Ventures of America Inc., Corona, CA), and the tensile bond strength was evaluated at a crosshead speed of 1.0 mm/min.

Statistical analysis

The null hypothesis was that bond strength would not be decreased by self-conditioner treatment and thermal loading, based on our results from a previous study[15] as well as the prevailing theoretical background. This means it was possible to conduct an a priori multiple comparison test, and thus, the null hypothesis was tested without taking the F-distribution into account. Specifically, the Tukey-Kramer multiple comparisons test was used without performing analysis of variance. An α value of 0.05 or less was considered statistically significant.

Observation of failure modes after tensile bond strength test

After the tensile bond strength test was performed, the test specimens were examined using a digital stereoscopic microscope (SZ61; Olympus Corp., Tokyo, Japan) at 10× magnification to look for the fol-
Observation of the adhesive interface

Samples prepared similarly to those for the microtensile bond strength test were subjected to the thermal cycle (10,000 cycles of 5°C for 30 s and 55°C for 30 s), and sectioned perpendicular to the adhesive interface using the IsoMet low-speed precision saw. The obtained sections were polished using silicon carbide waterproof abrasive paper sheets (up to 2,000 grit), and a 5-min ultrasonic cleaning was performed 3 times. The adhesive interface was observed in detail by scanning electron microscopy (VE-9800; Keyence Corp, Osaka, Japan) without sputter deposition.

Results

Microtensile bond strength

Measurements of microtensile bond strength and the results of statistical analysis are shown in Table 3 and Fig. 3. Results are presented as the mean (SD).

Bond to non-irradiated dentin:

The initial bond strength of the specimens without dentin surface treatments (the NT0 group) was 11.6 (3.3) MPa; the test could not be performed for those subjected to thermal loading (the TC NT0 group) because of the interfacial dissociation that occurred during test-sample preparation. In the Fe0 group, the initial bond strength was 23.5 (5.6) MPa, which was significantly reduced to 8.5 (2.3) MPa by the addition of TC (the TC Fe0 group, \( p < 0.05 \)). In the Pr0 group, the initial bond strength was 25.9 (4.8) MPa, and this was not significantly changed after the TC (31.5 (2.5) MPa in the TC Pr0 group, \( p > 0.05 \)). The bond strength was significantly higher in the TC Pr0 group than in the TC Fe0 group (\( p < 0.05 \)).

Bond to dentin subjected to irradiation at low output power:

The microtensile bond strength test could not be performed when dentin surface treatment was not performed (the NT50 group and the TC NT50 group) because of interfacial dissociation that occurred during sample preparation. The initial bond strength was 12.8 (4.6) MPa in the Fe50 group and 10.6 (4.2) MPa in the TC Fe50 group; the difference was not statistically significant (\( p > 0.05 \)). Similarly, the strengths in the Pr50 group and the TC Pr50 group were 26.8 (4.6) and 22.3 (6.4) MPa, respectively; the difference was not statistically significant (\( p > 0.05 \)). The effect of thermal loading was not observed in both the Fe group and the Pr group (\( p > 0.05 \)). The bond strength was significantly higher in the Pr50 group than in the Fe50 group and the TC Fe50 group (\( p < 0.05 \)).

Bond to dentin subjected to irradiation at low output power and finishing irradiation:

Trends were similar to those described in the above section. That is, microtensile bond strength test could not be performed when dentin surface treatment was not performed (the NT50+150 group and the TC NT50+150 group) because of interfacial dissociation that occurred during sample preparation. The bond strength was 10.0 (5.8) MPa in the Fe50+150 group and 5.1 (1.8) MPa in the TC Fe50+150 group, which were not significantly different (\( p > 0.05 \)). Similarly, the bond strength was 26.6 (3.2) MPa in the Pr50+150 group and 23.0 (6.9) MPa in the TC Fe50+150 group, which were not significantly different (\( p > 0.05 \)). The effect of thermal loading was not observed in the Fe group and the Pr group (\( p > 0.05 \)), and the bond strength was significantly higher under all experimental conditions in the Pr group than in the Fe group (\( p < 0.05 \)).
It is noteworthy that the microtensile bond strength of RMGI to the laser irradiated, acid-conditioned, and primed dentin with/without thermal loading (the Pr50, TC Pr50, Pr50+150 and TC Pr50+150 groups) was not significantly different from that to the non-irradiated, acid-conditioned and primed dentin (the Pr0 and TC Pr0 groups, \( p > 0.05 \)).

**Modes of failure observed after the microtensile bond strength test**

The frequency distribution of failure modes observed after the microtensile bond strength test in each group is shown in Fig. 4.

- **Bond to non-irradiated dentin:**
  - In the NT0 group, adhesive failure at the dentin–RMGI interface was confirmed in all test specimens, 20% of which had cohesive failure in RMGI. Interface separation occurred during specimen preparation for microtensile bond strength test in the TC NT0 group. In the Fe0 group, cohesive failure in RMGI was found in 80%, adhesive failure in 60%, and cohesive failure in dentin in 20% of the test specimens. Failure modes in the TC Fe0 group were similar to those in the Fe0 group; specifically, cohesive failure in RMGI was found in 90% and adhesive failure in 10% of the test specimens; no cohesive failure in dentin was observed. In the Pr0 group, cohesive failure in dentin was found in all test specimens, 80% of which had cohesive failure in RMGI. Failure modes in the TC Pr0 group were similar to those in the Pr0 group; that is, cohesive failure in dentin was found in 90%, cohesive failure in RMGI in 60%, and adhesive failure in 10% of the test specimens.
  - Thermal loading tended to increase the frequency of cohesive failure in RMGI in the Fe group and that in dentin in the Pr group.

- **Bond to dentin subjected to irradiation at low output power:**
  - Interfacial dissociation occurred during specimen preparation in both the NT50 group and the TC NT50 group. In the Fe50 group, cohesive failure in RMGI was found in 80%, adhesive failure in 50%, and cohesive failure in dentin in 40% of the test specimens. In the TC Fe50 group, adhesive failure was found in 80%, cohesive failure in RMGI in 60%, and cohesive failure in dentin with adhesive failure in 20% of the test specimens. In the Pr50 group, cohesive failure in RMGI was found in 80%, cohesive failure in dentin in 70%, and cohesive failure in dentin with adhesive failure in 30% of the test specimens. In the TC Pr50 group, cohesive failure in dentin was found in 80%, cohesive failure in dentin with adhesive failure in 50%, and cohesive failure in RMGI in 30% of the test specimens.
  - Thermal loading tended to increase the frequency of adhesive failure and mixed-mode failure (cohesive failure in dentin with adhesive failure) in both the Fe group and the Pr group.

- **Bond to dentin subjected to irradiation at low output power plus finishing irradiation:**
  - Interfacial dissociation occurred during specimen preparation in both the NT50+150 group and the TC NT50+150 group. In the Fe50+150 group, cohesive failure in RMGI was found in all test specimens; 50% had cohesive failure in dentin and 10% had adhesive failure. In the TC Fe50+150 group, cohesive failure in RMGI was found in all test specimens; 50% had cohesive failure in dentin and 10% had adhesive failure. In the TC Pr50+150 group, cohesive failure in dentin was found in 80%, cohesive failure in RMGI in 70%, and cohesive failure in dentin with adhesive failure in 30% of the test specimens.
Fe50+150 group, cohesive failure in RMGI was found in 90%, adhesive failure in 40%, and cohesive failure in both RMGI and dentin in 30% of test specimens. In the Pr50+150 group, cohesive failure in RMGI was found in 90%, cohesive failure in dentin in 70%, and adhesive failure in 30% of test specimens. In the TC Pr50+150 group, cohesive failure in RMGI was found in 80%, adhesive failure in 70%, and cohesive failure in dentin in 40% of test specimens.

Thermal loading tended to decrease the frequency of cohesive failure in dentin but to increase the frequency of adhesive failure in both the Fe and the Pr groups.

Discussion

We examined the initial bond strength (24 h after bonding) and bond durability (i.e., bond strength after thermal loading of 10,000 TCs) of RMGI to dentin that was irradiated with an Er:YAG laser at a low output power with/without finishing irradiation, and then, acid-conditioned using a solution of 10% citric acid and 2% ferric chloride, or similarly acid-conditioned and primed with hydrophilic monomers (4-MET and HEMA).

When the dentin was not irradiated, the beneficial effect of acid-conditioning was confirmed; the approximate initial bond strength of 11 MPa in the NT0 group vs 24 MPa in the Fe0 group. When dentin was irradiated (at a low output power with/without finishing irradiation), interfacial dissociation occurred in all specimens during test-specimen preparation when acid treatment was not performed (the NT50 group and the NT50+150 group). However, when acid-conditioning was performed (the Fe50 group and the Fe50+150 group); the approximate initial bond strength was 10 MPa in the Fe50+150 group and 13 MPa in the Fe50 group, the obtained bond strengths were in good agreement with our previous results13,14.

When thermal loading was applied without acid-conditioning, RMGI-dentin interfacial dissociation occurred in all specimens, irrespective of laser irradiation (i.e., the TC NT0 group, the TC NT50 group and the TC NT50+150 group). However, when non-irradiated dentin was acid-conditioned, the bond strength was measurable but significantly reduced after thermal loading (approximately 9 MPa in the TC Fe0 group). In contrast, the effect of thermal loading was negligible when dentin was irradiated at low output power with/without finishing irradiation; the bond strengths in the TC Fe50 group and the TC Fe50+150 group were similar to that in the TC Fe0 group.

However, failure modes varied in the TC Fe0 group, the TC Fe50 group and the TC Fe50+150 group. The effect of thermal loading was negligible and cohesive failure in RMGI was observed mainly in the TC Fe0 group, but adhesive failure between dentin and RMGI was often observed in the TC Fe50 group. This might be explained by interfacial strain caused by increased polymerization of RMGI after thermal loading16. The increased frequency of cohesive failure in dentin in the TC Fe50+150 group can be explained by strain in the denatured layer of dentin, rather than in the interface, because the denatured layer was thinner when finishing irradiation was performed13-15.

When priming was performed in addition to acid-conditioning on non-irradiated dentin, the initial bond strength did not change much (approximately 26 MPa in the Pr0 group), though significant increases in bond strength resulting from priming have been reported17. When priming was performed in addition to acid-conditioning on irradiated dentin irrespective of irradiation condition, the approximate initial bond strength was 27 MPa in both the Pr50 group and the Pr50+150 group, which was similar to that in the Pr0 group; this was in good agreement with the findings of our previous study14. Thermal loading did not affect the bond strength to acid-conditioned and primed dentin with/without irradiation, irrespective of irradiation condition.

In the Pr group, cohesive failure in dentin was found in most test specimens regardless of the thermal loading when irradiation was not performed. In contrast, cohesive failure in RMGI was found in nearly all test specimens when irradiation was performed, irrespective of irradiation condition; 70% had cohesive failure in dentin and the remaining 30% had adhesion failure. This was in contrast to the predominance of adhesion failure in test specimens in the Fe group. Thus, it is likely that, when the denatured layer was strengthened by acid-conditioning and priming, failure occurred at the interface between the normal dentin and the denatured layer after thermal loading16,18, resulting in marked en-
enhancement of the bond strength in the Pr group compared with the Fe group (Fig. 5).

In the Pr group, the bond strength to the irradiated dentin became similar to that to non-irradiated dentin for the following reasons: 1) low-molecular-weight hydrophilic resin (4-MET and HEMA) in the priming solution impregnated and reinforced the denatured layer, which was generated in the dentin after laser irradiation; 2) Fe ions in the acid-conditioning solution (a citric acid–ferric chloride solution), which was applied before priming, promoted polymerization of the impregnated resin and the resin layer at the interface, resulting in further reinforcement; 3) after the denatured layer was reinforced, the mechanical properties of normal dentin, the denatured layer, and the bonding material (RMGI) became similar, and failure occurred at the interface between the denatured layer and normal dentin rather than at the interface between the RMGI and the denatured layer during the tensile bond strength test (Fig. 619-21).

Taken together, the 3-step RMGI bonding system incorporating acid-conditioning using a solution of 10% citric acid and 2% ferric chloride followed by priming with hydrophilic monomers (e.g., 4-MET and HEMA), reinforces the denatured layer caused by laser irradiation, thereby improving the initial strength and durability of the bond to the irradiated dentin to a level similar to those of the bond to non-irradiated dentin.

This study demonstrated improved bonding to Er:YAG laser-irradiated dentin via reinforcement of the denatured layer and use of RMGI. However, the value of the bond strength thus achieved was still weaker than that achieved by conventional resin bonding systems. Therefore, further study is required to develop a system with performance similar to conventional resin bonding systems that can be applied to laser-irradiated dentin.

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Conflicts of Interest
The authors have declared that no COI exists.

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