Effect of Conditioners on Bond Durability of Resin Composite to Nd:YAP Laser-irradiated Dentin

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The aim of this study was to evaluate the effect of conditioners (sodium hypochlorite (NaOCl), Roth’s ethylenediaminetetraacetic acid (EDTA), and phosphoric acid) on shear bond strength and morphology of Nd:YAP laser-irradiated dentin. In particular, the key focus was on the bond durability between resin composite and treated dentin after being subjected to thermocycling in artificial saliva between 5°C and 55°C. Results indicated that the application of phosphoric acid to laser-irradiated dentin produced a bond strength comparable to those using NaOCl and EDTA. Further, dental tubules which were closed after laser irradiation opened following the treatment with conditioners. When subjected to 3,000 thermocycles, the mean shear strength of the samples treated by the three conditioners following laser irradiation ranged from 9.7 to 12.6 MPa with a reduction of 25-33% - a reduction rate lower than that obtained using acid etching alone (50%). Among the three conditioners tested, only phosphoric acid treatment demonstrated an enhanced effect on bond durability of laser-irradiated dentin.

Key words : Nd:YAP laser, Shear bond strength, Conditioner

INTRODUCTION

Durable adhesion of restorative material to dentin plays an undisputedly pivotal role in a successful dental restorative treatment. In particular for long-term restorative applications, many factors that affect bond strength must be taken into consideration – namely, physical and chemical properties of the restorative material, operating limitations of the restorative material, the intraoral environment, and occlusal forces1–4. To modify the complex dentinal structure so as to enhance the bond strength of resin composites to dentin, a variety of treatment techniques – such as phosphoric acid, self-etching systems, and dental lasers – have been developed5–9. Of this wide range of techniques, lasers have been used for caries prevention, caries removal, endodontic treatment, dentin hypersensitivity, and as an alternative to conventional acid-etching technique in clinical applications2–15.

Regarding dentin morphology after laser application, the following changes have been reported to take place: a melted and re-solidified surface, the presence of craters, cracks, and irradiation globules, and the occlusion or narrowing of dentinal tubules – all appreciably depending on the kind of laser used8,12–14,16. In terms of bond durability, laser pretreatment of dentin served to prepare a better surface for bonding of resin composite materials than the acid-etching method8. On the other hand, other studies indicated that laser treatment did not provide more superior dentin bonding7,9,16. Hence, in a bid to increase the bond strength of resin composites to laser-irradiated dentin, some studies have followed up laser irradiation with various treatments using conditioners15,17,18. In a study by Eguro et al.19, the application of phosphoric acid on Er:YAG laser-irradiated dentin surface significantly increased the bond strength, as compared to the bond strength obtained using laser irradiation alone.

It is well known that an ideal dentin bonding agent should possess these properties: good long-term adhesion to dentin, no microleakage between restorative material and dentin, storage stability, easy to use, tolerance of the oral environment, and unharmful to the dental pulp19–21. However, over time, the bonding agent hydrolyzes and degrades – leading to premature resin-dentin bond failure4,22. It was also reported that dentin bonding agent could not infiltrate the whole of the dentin layer decalcified by an etchant, thus causing demineralized dentin not infiltrated by resin to remain between the hybrid layer and intact dentin23,24. In light of a porous dentin zone that acts as a microleakage pathway beneath the resin-impregnated layer, Nakabayashi et al.25 therefore suggested that when using resin-based restorative materials with dentin bonding agent, the formation of a resin-infiltrated dentin layer would provide the most efficient dentin adhesion mechanism.

In a previous study16, we found that laser-irradiated dentin surface had a lower degradation rate in shear bond strength when subjected to thermocycling in artificial saliva as compared to...
conventional acid-etching treatment. Following the preliminary study, we set out to increase the bond strength of resin composite to Nd:YAP laser-irradiated dentin. To meet this objective in the present study, Nd:YAP laser-irradiated dentin was treated with NaOCl, EDTA, or phosphoric acid. Moreover, to our knowledge, limited information is available on the bond durability of resin composites to dentin treated by laser irradiation followed by secondary treatment. Against this background, a key focus of the present study was to evaluate the shear bond strength after thermocycling.

MATERIALS AND METHODS
A total of 100 freshly extracted caries-free human molars were collected and stored in 4°C physiological saline solution and used within one month after extraction. The root was mounted in epoxy resin and cut using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). After which, the coronal dentin surface was polished with 600-grit silicon carbide sandpaper under wet conditions. The dentin specimens were then randomly allocated among the designated groups, whereby two different treatments were carried out on the dentin surface: phosphoric acid etching or Nd:YAP laser irradiation.

Phosphoric acid etching
Thirteen samples were acid etched with 35% phosphoric acid gel of pH 0.9 (Scotchbond Etchant, 3M, St. Paul, MN) for 20 seconds, then rinsed with water for the next 10 seconds, and air-dried for two seconds. After which, the dentin samples were applied with Scotchbond MP Primer and dried gently for five seconds before light-curing Scotchbond MP Adhesive with visible light (QHL 75, Dentaply Caulk, Milford, DE, USA) of 575 mW/cm² for 20 seconds according to the manufacturer’s recommendations.

Nd:YAP laser irradiation
A total of 85 polished dentin surfaces were irradiated by a Nd:YAP laser system (Lokki, Vienne, France) based on yttrium aluminum perovskite at 300 mJ and 10 Hz for 60 seconds according to manufacturer’s recommendations. It had a wavelength of 1.34 μm and pulse duration of 150 μs. Laser treatment was conducted using an optical fiber with a diameter of 320 μm. As it is an invisible light, a red He-Ne laser beam scanned the moist surface within a limited circle of 3-mm diameter in a perpendicular direction at a distance of 10 mm for about one minute per sample.

Secondary treatment following laser irradiation
Seventy-two samples of 85 teeth in the laser-irradiated group were randomly divided into three subgroups with 24 samples per subgroup. The remaining ones were regarded as laser irradiation alone. These three subgroups proceeded respectively with the designated secondary treatment using 1.5% NaOCl (pH 9.5) (Shimakyu, Osaka, Japan) for two minutes, EDTA pH 8.0 (Roth’s ethylenediaminetetraacetic acid, Roth International, Chicago, IL) for one minute, or phosphoric acid for 20 seconds. EDTA was a mixture of 17 g disodium ethylenediaminetetraacetate, 0.84 g cetyl-trimethyl ammonium bromide, and 9.25 mL of 5 N sodium hydroxide solution in 100 mL distilled water. After which, treated dentin was rinsed with water for 20 seconds and air-dried for two seconds.

Shear bond strength test
Once dentin surfaces were prepared accordingly as described above, Scotchbond MP Primer and Adhesive were applied in the same way as the phosphoric acid etching only group. After which, TetricFlow resin (Vivadent, Liechtenstein, Germany) was bonded to dentin samples via a plastic mold and then light-cured in three layers to form a resin cylinder of 3 mm in height and 3 mm in diameter using QHL 75 at 575 mW/cm². All specimens were stored in distilled water at 37°C for 24 hours before being subjected to thermocycling and shear bond strength.

For thermocycling, 11 of 24 samples in each subgroup (i.e., laser irradiation followed by secondary treatment with conditioner) proceeded with 3,000 thermocycles in artificial saliva after bonding to light-cured resin. Formation of the artificial saliva was similar to the composition of inorganic ions in saliva, consisting of 0.4 g NaCl, 0.690 g NaH₂PO₄·H₂O, 0.4 g KCl, 0.005 g Na₂S·9H₂O, 0.795 g CaCl₂·H₂O, 0.300 g KSCN, and 1.000 g urea in 1 L distilled water. Each cycle consisted of a 15-second dwell time in each artificial saliva bath of 5°C and 55°C with 3-second transfer time using a thermocycling machine.

For shear bond strength test, specimens with and without thermocycling were secured onto a custom fixture of a Bencor Multi-T testing device (Danville Engineering Co., Danville, CA). Shear bond strength was performed in air at room temperature using an EZ Test machine (Shimadzu, Kyoto, Japan), which was equipped with a chisel-shaped rod to deliver the shear stress. Specimens were aligned such that the rod was against and parallel to the flat, prepared resin-dentin bonding site. The bonded resin-dentin samples sustained continuous loading at a crosshead speed of 0.5 mm/min until fracture.

Statistical analysis
One-way ANOVA method was used to evaluate the statistical significance of mean shear bond strength. A Tukey’s post-hoc multiple comparison test was used to determine the significance of the deviations in bond strength among the test groups after different
treatments. Level of statistical significance was set at $P=0.05$. Eleven specimens from each subgroup were tested.

Microstructural observation
Two untreated dentin samples and two samples of each type of treated dentin surface were desiccated and coated with Pt-Ag film. The dentinal microstructure was observed under a field-emission scanning electron microscope (FESEM, S-4200, Hitachi, Tokyo, Japan). Cross-sections of the fractured specimens after shear test were also observed with this FESEM. Specimens for cross-sectional microscopy were prepared by mounting the fractured specimens in epoxy resin and cut perpendicular to the fractured dentin surface by using a low-speed diamond saw, followed by polishing with 0.3-$\mu$m alumina powder.

RESULTS
Shear bond strength
Table 1 lists the shear bond strength results of the resin composite to all types of treated dentin. In terms of pretreatment effect, mean bond strength between resin composite and acid-etched dentin was significantly higher ($P<0.05$) than with Nd:YAP laser irradiation only. In terms of secondary treatment effect, NaOCl-, EDTA-, and phosphoric acid-treated dentin samples showed no significant differences ($P>0.05$) in mean bond strength before thermocycling. Interestingly, the bond strength difference between phosphoric acid-etched samples with and without laser irradiation was not significant ($P>0.05$).

For the shear bond strength results of acid-etched only and laser-irradiated only dentin samples after 3,000-cycle thermocycling, they were already given in a previous study. Therefore, in this study, only laser-irradiated samples with secondary treatment were evaluated to compare with those without thermocycling (Table 1). After alternating between cold and hot saliva baths for 3,000 cycles, the mean shear bond strength of resin to laser/NaOCl-treated dentin declined significantly ($P<0.05$) with a 30% reduction. In EDTA-treated group, the decrease in shear bond strength after thermocycling was 33%. As for phosphoric acid-treated group, the decrease in shear bond strength was a mere 25%.

Morphology of treated dentin
Fig. 1 shows the surface morphologies of untreated, phosphoric acid-etched, and laser-irradiated dentin. Depending on the pretreatment method, the surface morphologies varied in terms of the amount of opened dentinal tubules and the size of the openings. For the untreated dentin, the dentinal tubules had a smaller opening and the tubular openings were partially sealed. After conventional phosphoric acid treatment, the dentin surface appeared to be smooth and contoured. Besides, the dentinal tubules were opened and enlarged to approximately 5$\mu$m in diameter. In contrast, Nd:YAP laser irradiation left most of the dentinal tubules sealed, in addition to the scraps. As shown by arrows in Fig. 1(c), laser-induced cracks appeared widely on the sealed surface.

With secondary treatment following laser irradiation, the originally closed dentinal tubules were now clearly visible on the dentin surface (Fig. 2). Tubular openings with NaOCl and EDTA treatments were smaller in size when compared with those of phosphoric acid treatment. Besides, NaOCl and EDTA yielded a smaller number of opened dentinal tubules than phosphoric acid. When comparing between NaOCl and EDTA treatments, the former treatment gave rise to dentinal tubules with openings slightly larger than those of normal dentinal structure, but nonetheless smaller than those produced by EDTA.

Fig. 3 shows the resin-dentin interface representative of fractured specimens treated with phosphoric acid alone. Cross-sectional SEM micrograph revealed infiltration of adhesive resin but which did not penetrate deep enough, thus leaving voids as indicated by arrows. On the contrary, for the three dentin samples treated with conditioners following laser irradiation (Fig. 4), the adhesive resin seemed to seal the dentinal tubules — although most of the tubular openings were already occluded by laser irradiation alone.

Table 1 Mean shear bond strengths and standard deviations (SD) of treated dentin before and after thermocycling

<table>
<thead>
<tr>
<th>Dentin treatment</th>
<th>Mean±SD (MPa) Before thermocycling</th>
<th>Mean±SD (MPa) After thermocycling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphoric acid etching only</td>
<td>18.4±5.4$^a$</td>
<td>9.1±1.4$^{a,c}$</td>
</tr>
<tr>
<td>Laser irradiation only</td>
<td>13.5±2.7$^b$</td>
<td>8.1±1.7$^{a,c}$</td>
</tr>
<tr>
<td>Laser irradiation + NaOCl etching</td>
<td>13.9±2.9$^b$</td>
<td>9.7±1.8$^c$</td>
</tr>
<tr>
<td>Laser irradiation + EDTA etching</td>
<td>14.9±2.3$^{a,b}$</td>
<td>10.0±1.6$^c$</td>
</tr>
<tr>
<td>Laser irradiation + phosphoric acid etching</td>
<td>16.7±4.5$^{a,b}$</td>
<td>12.6±2.4$^{a,d}$</td>
</tr>
</tbody>
</table>

Number of samples is 11 in each subgroup.

Mean values followed by the same superscript letter re not significantly different ($P>0.05$) according to Tukey’s post-hoc multiple comparisons.

$^a$These data are taken from Reference 16.
Fig. 1 SEM micrographs of untreated (a), phosphoric acid-etched (b), and laser-irradiated (c) dentin surfaces. The arrows indicate the laser-induced cracks.

Fig. 2 SEM micrographs of laser-irradiated dentin surfaces followed by NaOCl (a), EDTA (b), and phosphoric acid (c) treatments.

Fig. 3 SEM micrograph of resin-dentin interface for phosphoric acid-etched only sample. The arrows indicate the voids that the resin tags did not reach.
DISCUSSION

Generally, to increase dentin adhesion, both primer and adhesive agent have to penetrate dentin to form a micromechanical bond with collagen fibers and with microporosities in dentinal tubules as well as create a chemical bond to dentin. With conventional acid-etching method, a microporous dentin surface was created (as shown from SEM micrographs in Fig. 1) because phosphoric acid removed the smear layer and caused the dentinal tubules to be opened and enlarged. However, aggressive acids that provide etching can denature collagen fibers and demineralize a thicker layer of dentin — into which adhesive resins cannot penetrate thoroughly to reach the full depth, thus leaving a porous zone as a microleakage pathway beneath the resin-infiltrated layer. In addition, the tubules were most likely filled with water, thus preventing resin infiltration. With insufficient resin infiltration and water-filled dentinal tubules, these factors hence accounted for the unfavorable adhesion results of resin composites to phosphoric acid-etched dentin when subjected to thermocycling, as reported in our previous study and in other reports.

Laser uses heat to melt the dentinal surface. At higher doses, however, thermal effects of lasers may lead to microcavities and/or cracks. Heat accumulation caused by laser irradiation makes the organic components within dentin to be evaporated and melts the inorganic components. Following which is crystallization, whereby a new surface structure similar to enamel is formed. After Nd:YAP laser irradiation in this study, we observed that the dentinal tubules were almost sealed without any exposed, open tubules. Further, Nd:YAP laser irradiation produced a mean shear bond strength of 13.5 MPa — which was comparable to the 12.9 MPa obtained by Visuri et al. using Er:YAG laser treatment on dentin, but significantly lower than that of acid-etched dentin (18.4 MPa). Researches indicated that laser treatment might enhance the shear bond strength of resin composites to tooth substrates in some situations, but otherwise unsatisfactory in other cases. This was because laser-treated surfaces were covered with a mixed layer or that fractured tooth structures covered the bonding surface. Additionally, irregularities and fissures on dentinal surface after laser-irradiation-only pretreatment were insufficient to provide resin adhesives with good adhesion.

Structure on the outer dentin surface and the closing of most of the dentinal tubules in Nd:YAP laser-treated samples were most likely caused by a very thin surface layer of unknown composition. This thin, laser-induced layer could be modified by subsequent treatment with a conditioner, such that the dentinal tubules were partially or completely opened again. SEM micrograph of phosphoric acid etching following laser irradiation clearly indicated an actual enlargement of tubular diameter in com-

Fig. 4 SEM micrographs of the resin-dentin interface after laser irradiation only (a); followed by treatment with NaOCl (b), EDTA (c), and phosphoric acid (d).
comparison to the other two samples treated with NaOCl and EDTA, thus lending support to the conjecture that phosphoric acid might increase the micromechanical interlocking of resin composites to dentin. Indeed, this conjecture was further substantiated by a substantial increase in shear bond strength of laser-irradiated dentin followed by phosphoric acid etching (Table 1), which was in agreement with findings by Eguro et al. In the same vein, it is not difficult to understand that the bond strength of EDTA-treated sample (14.9 MPa) was slightly higher than that using NaOCl treatment (13.9 MPa) due to the difference in surface structure. Sodium hypochlorite (NaOCl) is a nonspecific proteolytic agent that effectively removes organic dentinal components, changes their composition, increases wettability, and enlarges tubules near the outer dentin surface. EDTA, on the other hand, is likely to be capable of removing both the organic and inorganic substances in dentin.

It is now established that infiltration of resin adhesives into dentin is a key factor to achieving a favorable resin bonding. While it remained unclear whether resin adhesives were able to thoroughly penetrate the dentinal tubules in dentin specimens treated by laser irradiation followed by secondary treatment, the surface structure and sealed dentinal tubules caused by Nd:YAP laser irradiation might provide an effective resistance against etching by the three chemical agents — thus resulting in a lower depth of demineralized dentin zone to be infiltrated. White et al. suggested that the thin laser-induced layer could effectively preclude infiltration of resin and/or conditioners into the dentinal tubules, and thus provided sealer penetration resistance similar to that offered by a smear layer.

High bond strength between restorative resin and dentin plays an important role in the long-term performance of restorations in the oral environment. Nonetheless, it is not the sole requirement because bond durability is also of tantamount importance. Thermocycling has been recognized as a useful method to evaluate the sealing ability of resin-bonded restorative materials to tooth substrates. In a recent study, we found that Nd:YAP laser-irradiated dentin surfaces had a lower reduction rate (39%) in shear bond strength after thermocycling for 3,000 cycles in artificial saliva at 5°C and 55°C as compared to the conventional acid-etching treatment (50%). After thermocycling for 3,000 cycles in artificial saliva in this study, laser-irradiated dentin samples with three different secondary treatments showed a significant decrease in mean shear bond strength ranging from 25 to 33%. During thermal cycling, the specimens were subjected to thermal changes and also to additional exposure to water that resulted in degradation of resin-dentin bond. De Munck et al. found that upon direct exposure to water for four years, resin-dentin bonds produced by two-step total-etch adhesives were significantly affected. Further, temperature fluctuations ranging from 5°C to 55°C might cause gap formation and microleakage at the tooth-resin interface, and thereby induced surface stresses because of high thermal gradients near the surface — eventually leading to degradation of resin-dentin bond. Indeed, reduction in bond strength could be due to the effect of hydrolysis at the interface between bonding resin and hybrid layer. However, a combined protocol comprising both laser irradiation and conditioner treatment seemed to produce a more stable bonding to dentin — thereby illustrating the need for an adhesive agent to be accompanied with a conditioning agent. On this note, it was evident that phosphoric acid treatment indeed had an enhanced effect on the bond durability of laser-irradiated dentin.

CONCLUSIONS

This study investigated the effect of treating laser-irradiated dentin with several conditioning agents prior to bonding with a resin-based adhesive. This combined protocol of laser irradiation followed by conditioner treatment caused the dentinal tubules which were closed after laser irradiation to be open. Moreover, dentin surfaces with secondary treatment exhibited a lower reduction rate in shear bond strength than that by conventional acid etching alone after 3,000-cycle thermocycling in artificial saliva. Although shear bond strength declined after thermocycling, Nd:YAP laser irradiation followed by phosphoric acid etching was considered an effective treatment due to a lower decrease in shear bond strength. However, this present method of combining laser irradiation with a conditioning agent is not recommended for clinical use yet, although it does seem to suggest a new type of resin retention mechanism.

REFERENCES


