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Effect of Er,Cr:YSGG Laser on the Microtensile Bond Strength of Two Different Adhesives to the Sound and Caries-affected Dentin

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Clinical Relevance

The microtensile bond strength of a three-step etch-and-rinse and a two-step self-etch adhesive is not negatively affected by Er,Cr:YSGG laser irradiation in sound and caries-affected dentin.

SUMMARY

Objective: This study examined the effect of Er,Cr:YSGG laser irradiation on the microtensile bond strength (µTBS) of a three-step etch-and-rinse and a two-step self-etch adhesive to sound and caries-affected dentin.

Methods: Sixteen freshly extracted human molars with occlusal dentin caries were used. The caries lesion was removed by one of the following methods: conventional treatment with burs or Er,Cr:YSGG laser (Waterlase MD, Biolase). The adhesive systems (AdheSE, Ivoclar Vivadent and Scotchbond Multi Purpose, 3M ESPE) were applied to the entire tooth surface according to the manufacturers' instructions. Resin composites were applied to the adhesive-treated dentin surfaces and light-cured. Each tooth was sectioned into multiple beams with the "non-trimming" version of the microtensile test. The specimens were subjected to microtensile forces (BISCO Microtensile Tester, BISCO). The data was analyzed by three-way ANOVA and independent *t*-tests (*p*=0.05).

Results: Er,Cr:YSGG laser irradiation exhibited similar µTBS values compared to that of conventional bur treatment, regardless of the adhesive

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system and type of treated dentin. The self-etch system revealed lower µTBS values, both with conventional and laser treatment techniques, compared to the etch-and-rinse adhesive in sound and caries-affected dentin (*p*<0.05).

Conclusion: Er,Cr:YSGG laser irradiation did not negatively affect the bonding performance of adhesive systems to sound and caries-affected dentin.

INTRODUCTION

Since the development of effective adhesive systems, minimum-intervention dentistry procedures have been designed as much as possible toward the conservation of tooth structure by eliminating carious tissues, while avoiding the removal of sound tooth substrate.[1] Using this approach, caries-affected dentin, which can be remineralized following restoration, is left during caries removal in cavities. Not expanding the cavity for prevention or macromechanical retention is performed and only bacterial contaminated, denaturated and caries-infected dentin is removed.[1]

The commonly used methods for restorative procedures are rotary instrumentation with burs at low and high speeds.[2-3] In addition to some of the advantages of these techniques, such as speed and low cost, they can cause patient discomfort and the need for local anesthesia. These disadvantages have led to the development of new technologies for dental hard tissue preparation and caries removal, such as laser irradiation.[4]

When different techniques are proposed, not only their efficiency, but also their ability to prepare the surfaces adequately for the subsequent bonded restorations, have to be considered. Different dental hard tissue preparation and caries removal techniques create distinct tooth substrates to be restored. For adhesive dentistry, the effectiveness of adhesive systems is crucial and mostly depends on the surface characteristics of prepared dental substrate. Thus, it is important to analyze the effect of different hard tissue preparation and caries removal techniques on the bond strength of adhesive systems.

Although several types of laser systems, which can cut dental hard tissues efficiently, have been introduced, erbium lasers have been considered to be the most promising laser device. Different erbium laser wavelengths, such as Er,Cr:YSGG and Er:YAG, have a similar major absorption band of water; however, Er,Cr:YSGG (2.78 µm) is better absorbed by hydroxyapatite than Er:YAG (2.98 µm).[5-6] Er,Cr:YSGG laser cuts dental hard tissue with the help of its laser-powered hydrokinetic system, which operates with a pulsed beam and sapphire tip bathed in a mixture of air and water spray. Previous studies have shown that micro-explosions formed during laser ablation are able to remove hard tissue particles from the irradiated areas, resulting in a rough surface with open dentinal tubules and without a smear layer.[5,7] However, contradictory results have also been reported in the literature regarding the bonding effectiveness of adhesive systems to laser-irradiated dentin.[8-10] Some studies demonstrated that the bond strength of resin composite to Er:YAG laser-irradiated dentin increased when the surface was acid-etched before bonding.[11]

With self-etch adhesive systems, which include weak acidic monomers, the dentinal smear layer is no longer completely eliminated. These adhesive systems produce very fine hybrid layers with an area composed of a hybridized smear layer and another area composed of hybridized subjacent dentin.[12] After laser irradiation, no smear layer is created.[13] When using a self-etch adhesive system on irradiated dentin, the acidic monomers act on a smear layer dentin-free surface. A number of studies examined the effects of laser irradiation on the bonding efficiency of self-etch adhesives to dentin, especially caries-affected dentin.[14-15] There is still a question as to whether or not laser irradiation interferes with the bonding of self-etch systems to dentin.

The current study examined the effect of Er,Cr:YSGG laser irradiation on µTBS in a three-step etch-and-rinse and a two-step self-etch adhesive to sound and caries-affected dentin. The hypotheses to be tested were that (a) Er,Cr:YSGG laser irradiation does not negatively affect the bonding performance of adhesive systems to sound and caries-affected dentin and (b) the two-step self-etch system produces lower bond strengths than the three-step etch-and-rinse adhesive in sound and caries-affected dentin.

METHODS AND MATERIALS

Tooth Type and Preparation

Sixteen freshly extracted human molars with occlusal dentin caries extending through the dentin were used in the current study. The extracted teeth were cleaned thoroughly to remove both hard and soft deposits, they were then stored at 4°C in a saline solution containing a few crystals of thymol until used. The occlusal caries lesions of the teeth were exposed by removing the occlusal enamel and superficial dentin using a slow-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling. The teeth were randomly assigned to two groups according to the treatment method: conventional treatment with burs or Er,Cr:YSGG laser (Waterlase MD, Biolase Technology Inc, Irvine, CA, USA) treatment. In the conventionally-treated samples, the carious tissue was removed using a round carbon-steel bur mounted in a contra-angle slow-speed handpiece with air as the coolant. To form the sound dentin groups, the entire normal dentin surrounding the carious tissue was also treated with these burs without forming cavities. For the laser-treated samples, an Er,Cr:YSGG laser was employed with S75/750 µm tips. A power of 2 W (65% air, 55% water) with 25 Hz was used for caries removal and 4 W (70% air, 30% water) with 25 Hz was used for sound dentin in a focused mode at 1-1.5 mm focal distance.

Removal of the carious tissue was guided by the combined criteria of the visual and tactile examination (by probing with an explorer) and staining with a caries disclosing solution (Caries Detector, Kuraray, Osaka, Japan). Dark pink to red-stained dentin was classified as caries-infected dentin, while discolored dentin no longer stained by caries detector solution was classified as caries-affected dentin. This removal resulted in an apparently hard caries-affected dentin surface in all specimens. After preparations, the teeth were rinsed with distilled water and air-dried. Each treatment group was further divided into two subgroups (n=4) according to the adhesive systems to be applied. The caries-affected and sound dentin of the cavities were bonded with either a three-step etch-and-rinse (Scotchbond Multi Purpose, 3M ESPE, St Paul, MN, USA) or a two-step self-etch (AdheSE, Ivoclar Vivadent, Schaan, Liechtenstein) adhesive system. Each adhesive was used according to the manufacturer's instructions (Table 1). A block of resin composite (Clearfil AP-X, Kuraray) with a height of 4 mm was built-up on the treated surface in two 2-mm thick increments. Light curing was performed using a halogen curing unit (Optilux 501, Kerr/Demetron, Danbury, CT, USA) with a light output exceeding 600 mW/cm^2. The teeth were stored in distilled water at 37°C for 24 hours.

Microtensile Test Procedures

Microtensile testing (µTBS) was undertaken using the non-trimming technique that was first described by Shono and others.[16] Each tooth was sectioned with a slow-speed saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling into multiple 0.9 x 0.9 mm beams. Four teeth for each bonding system were used. Standard five beams for both caries affected and sound dentin were obtained from each tooth. Twenty beams were tested for each subgroup and each bonding agent. At the time of trimming, selection of the testing region, such as caries-affected or normal dentin, was carefully performed by visual observations. The beams of the caries affected dentin were obtained just above the discolored affected dentin, and the beams taken from the dentin surface, which was at least a distance of 1 mm away from the discolored affected dentin, were used for the evaluation of µTBS in sound dentin. The area between the sound and caries-affected dentin was not used for obtaining beams. However, since this was done visually, it was not always possible to select regions that were 100% caries affected or normal dentin. Thus, all de-bonded species were re-evaluated using a light microscope during evaluation of the fracture type to confirm the type of bonded dentin. The caries-affected dentin is transparent in transmitted light and light brown in reflected light. Normal dentin is opaque in transmitted dentin and white in reflected dentin. Samples that showed mixtures of sclerotic and normal dentin were planned to be excluded from the current study. However, no sample with mixtures of sclerotic and normal dentin was determined. The specimens were then attached to a Bencor Multi-T testing apparatus (which was modified by Bernard Ciucchi, Danville Engineering Co, Danville, CA, USA) with cyanoacrylate adhesive (Zapit, DVA, Anaheim, CA, USA), then subjected to tensile forces in a microtensile testing machine (Microtensile Tester, BISCO, Inc, Schaumburg, IL, USA) at a crosshead speed of 1 mm/minute. The cross-sectional area at the site of failure was measured to the nearest 0.01 mm with a digital caliper (Model CD-6BS; Mitutoyo, Tokyo, Japan), from which the µTBS was calculated and expressed in MPa.

After microtensile testing, the fracture surfaces of all specimens were examined using a stereomicroscope (LG-P52, Olympus Co, Tokyo, Japan) to determine the mode of failure at 50x magnification. Failures were classified as adhesive (interfacial failure), cohesive in dentin, cohesive in resin (including failures either within the resin composite or adhesive layer) or mixed.

Table 1: *Composition and Application of the Adhesives Used*

Adhesive	Composition (Batch #)	Application Procedure
Scotchbond Multi Purpose (3M ESPE, St Paul, MN, USA)	**Etchant**: 37.5% phosphoric acid (Lot: 6 HH) **Primer**: aqueous solution of HEMA, polyalkenoic acid copolymer (Lot: 6 BB)	Apply the etchant for 15 seconds; rinse for 15 seconds, gently air dry for 10 seconds. Apply one coat of primer, leave undisturbed for 20 seconds, gently air dry for 5 seconds.
	Bond: Bis-GMA, HEMA, dimethacrylates and initiators (Lot: 6 PM)	Apply a single coat of adhesive and light cure for 20 seconds.
AdheSE (Ivoclar Vivadent, Schaan, Liechtenstein)	**Primer**: PPAA, dimethacrylate, initiators and stabilizers and water (LOT: K08200)	Apply for 30 seconds (15 seconds brushing), disperse excess with a strong stream of air.
	Bond: HEMA, dimethacrylate, silicon dioxide, initiators and stabilizers (LOT: K10657)	Apply for 20 seconds, blow to a thin layer. Light cure for 10 seconds.

Abbreviations—BisGMA: bisphenol A diglycidylmethacrylate; BPDMA: biphenyl dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; PPAA: phosphoric acid acrylate.

Statistical Analysis

Statistical analysis was carried out with the SPSS 13.0 software system. The µTBS data of the groups were statistically analyzed using three-way ANOVA and independent *t*-tests. The fracture modes of samples were compared by the Chi-square test. The level of significance was determined to be $p<0.05$ for all tests.

RESULTS

The mean and standard deviation of µTBS for the test groups are shown in Table 2. The interaction Caries removing method * Adhesive system * Type of treated dentin was statistically insignificant (Table 3). The interactions Caries removing method * Adhesive system, Adhesive system * Type of treated dentin and Caries removing method * Type of treated dentin were also insignificant (Table 3). The Er,Cr:YSGG laser irradiation exhibited similar µTBS values compared to that of conventional bur treatment, regardless of the adhesive system and type of treated dentin. The two-step self-etch system (Scotchbond Multi Purpose) revealed lower µTBSs values compared to the etch-and-rinse adhesive (AdheSE) both with conventional and laser treatment techniques in sound and caries-affected dentin ($p<0.05$).

The fracture modes of the groups are shown in Table 4. Significant differences were found among the study groups in terms of fracture modes ($p<0.05$). The groups using Scotchbond Multi Purpose revealed different frac-

Table 2: *µTBS Values (mean ± sd) in MPa*

	Groups	µTBS
I	Er,Cr:YSGG + SBMP + Caries-affected dentin	17.71 ± 3.78
II	Bur treatment + SBMP + Caries-affected dentin	16.33 ± 6.76
III	Er,Cr:YSGG + SBMP + Sound dentin	18.25 ± 5.5
IV	Bur treatment + SBMP + Sound dentin	17.32 ± 6.1
V	Er,Cr:YSGG + AdheSE + Caries-affected dentin	9.96 ± 4.55
VI	Bur treatment + AdheSE + Caries-affected dentin	9.99 ± 4.07
VII	Er,Cr:YSGG + AdheSE + Sound dentin	9.59 ± 2.9
VIII	Bur treatment + AdheSE + Sound dentin	11.38 ± 5.81

Abbreviations—SBMP: Scotchbond Multi Purpose

Table 3: *Three-way ANOVA Results*

		Sum of Squares	df	Mean Square	F	Sig
Main Effects	Caries removing method	64.364	1	64.364	2.139	0.146
	Adhesive system	1335.642	1	1335.642	44.396	0.000
	Type of treated dentin	2.416	1	2.416	0.080	0.777
2-way Interactions	Caries removing method * Adhesive system	80.656	1	80.656	2.681	0.104
	Adhesive system * Type of treated dentin	92.143	1	92.143	3.063	0.082
	Caries removing method * Type of treated dentin	28.174	1	28.174	0.936	0.335
3-way Interactions	Caries removing method * Adhesive system * Type of treated dentin	0.163	1	0.163	0.005	0.941

Statistically insignificant (p>0.05)

Table 4: *The Fracture Modes of Tested Groups*

	Groups	Adhesive	Mix	Cohesive in Dentin	Cohesive in Resin
I	Er,Cr:YSGG + SBMP + Caries-affected dentin	6	11	2	1
II	Bur treatment + SBMP + Caries-affected dentin	9	9	2	0
III	Er,Cr:YSGG + SBMP + Sound dentin	7	10	1	2
IV	Bur treatment + SBMP + Sound dentin	6	10	2	2
V	Er,Cr:YSGG + AdheSE + Caries-affected dentin	11	7	2	0
VI	Bur treatment + AdheSE + Caries-affected dentin	12	6	1	1
VII	Er,Cr:YSGG + AdheSE + Sound dentin	12	4	3	1
VIII	Bur treatment + AdheSE + Sound dentin	10	8	2	0

Abbreviations—SBMP: Scotchbond Multi Purpose ture patterns when compared with the AdheSE groups ($p<0.05$). The number of adhesive failures in the AdheSE groups was higher than the other groups.

DISCUSSION

Dentinal surfaces prepared with erbium lasers have significantly different characteristics from those prepared with conventional bur instruments. The literature has shown that surfaces irradiated by Er:YAG and Er,Cr:YSGG lasers displayed rough and clean areas without debris, with most of the dentinal tubules visible and wide open. The peritubular dentin was protruding from the surrounding intertubular dentin due to its higher mineral and lower water content.[17] This characteristic is supposed to favor the bond strength of resin-based materials to dentin when laser systems are used.[18-19] However, the results of previous studies were contradictory; some studies have demonstrated a decrease in bond strength values on laser-treated surfaces when compared to surfaces prepared by conventional bur instruments.[8,9,20-21] One of the reasons for the adverse effects on dentin adhesion with lasers might be that the erbium lasers could not selectively remove hydroxyapatite crystallites without having a harmful effect on the collagen fiber network. Ceballos and others[8] demonstrated a 3-4 µm altered dentin subsurface beneath, where collagen fibrils appeared to have lost cross-bonding and were fused together, eliminating interfibrillar spaces. Therefore, laser irradiation on the dentinal substrate can cause not only consequences to the collagen fibers but also to the quality of the mineral content of this substrate. In addition, microcracks were observed in most laser irradiated samples, indicating surface damage caused by laser irradiation.[9] Cardoso and others[22] reported that morphological alterations produced by Er,Cr:YSGG laser-irradiation adversely influenced the bonding effectiveness of adhesives to dentin. Some studies present the acid resistance of dentin by laser, reporting the possibility of erbium lasers to diminish the solubility of irradiated dentin when immersed in an acid solution.[23] In order to compensate for the negative effect of erbium lasers on adhesion to dentin, some investigators proposed the application of acid etching after adhesive procedures with laser irradiation.[24] In the current study, after laser irradiation, an acidic primer or a phosphoric acid was used during bonding procedures. The use of either an intermediary strong acidic primer or a phosphoric acid seemed capable of removing this modified surface layer, as the µTBS obtained from the current study did not show a significant difference between the Er,Cr:YSGG laser and conventional bur treatment method regardless of the adhesive used and irradiated dentin type.

Carious dentin consists of two layers, the outer necrotic, highly infected layer and the inner, less infected, demineralized but potentially repairable layer.[25] In clinical situations, the bonding surface most frequently encountered after caries excavation consists of caries-affected dentin.[26] Bonding to normal dentin with different adhesives has shown bond strengths significantly higher than those to caries-affected dentin.[27-29] However, in a study by Sengun and others,[30] these authors found that both etch-and-rinse and self-etch bonding systems were successful on normal and caries-affected dentin except for one-bottle systems. After the mechanical preparation of a cavity with rotating or manual instruments, an amorphous smear layer of organic and inorganic debris is formed on the surface of dentin. This smear layer results in a weaker resin infiltration. In order to obtain an adequate bond to dentin, this smear layer is initially removed or treated prior to placement of the restoration by a variety of methods, such as acid-etching or laser irradiation, allowing more resin infiltration into the dentin tubules.[31] In the current study, Scotchbond Multi Purpose revealed similar performance in caries-affected and sound dentin regardless of treatment methods. Likewise, AdheSE did not demonstrate any significantly different performance in caries-affected and sound dentin, regardless of treatment methods. This was probably due to the stronger demineralization effect of the phosphoric acid etching agent of Scotchbond Multi Purpose, the intermediary strong etching pattern of AdheSE and the laser's effective smear layer removing and etching capacity.

However, the intermediary strong two-step self-etch adhesive revealed lower µTBS data than the three-step etch-and-rinse adhesive both in caries-affected and sound dentin with both treatment methods. In the literature, the quality of adhesion to dentin with self-etch adhesives mainly depends on the type of adhesive system. While some authors reported favorable laboratory results with mild two-step self-etch adhesives,[32-34] others found higher tensile and bond strength results with etch-and-rinse adhesives compared to intermediary strong self-etch systems.[35-38] Neelima and others[37] evaluated the µTBS of Single Bond and AdheSE and obtained higher values with Single Bond. Atash and Van den Abbeele[38] reported higher bond strengths with AdheSE compared to Scotch Bond 1. The vast variability between performances of the different self-etch adhesives can be attributed to their different functional monomers with different properties: acidity, hydrolytic stability and chemical interaction capacity.[31] Intermediary strong self-etch adhesives exhibit deeper hybridization. However, the thickness of the hybrid layer and the presence of resin tags do not particularly influence the bonding performance, and chemical interaction between the monomers and hydroxyapatite may be more important.[34] Some monomers, such as 10-MDP and 4-MET, can chemically bond with calcium in hydroxyapatite, and the better *in vitro* and *in vivo* performance of some mild self-etch adhesives was also attributed to these monomers in the literature.[39] The functional monomer of the intermediary strong two-step self-etch adhesive used in the current study has no ability to chemically bond, and its lower bonding efficiency may be the result of the lack of this chemically bonding process. Furthermore, the higher number of adhesive failures in the groups treated with AdheSE may have been caused by the lower bonding effectiveness of this adhesive.

The current *in vitro* study was performed 24 hours after polymerization of the resin, and none of the other aging methods (such as aging by thermo-cycling or occlusal loading) was used during the experimental procedures. However, the longevity of adhesive bonds also has to be considered during the evaluation of their bonding effectiveness. Most studies reported significant decreases even after relatively short storage periods, thermo-cycling and occlusal loading.[40-42] A decrease in bonding effectiveness over time is supposed to be caused by degradation of the interface components by hydrolysis. The number of investigations relating the effect of aging procedures on the bonding performance of adhesives to irradiated dentin is limited. do Amaral and others[43] examined the microtensile bond strength of an etch and rinse adhesive to Er:YAG-prepared dentin after long-term storage and thermocycling. They reported that performance of the tested adhesive system to Er:YAG-laser irradiated dentin was negatively affected after one-month's water storage and 2,000 thermocycles, while adhesion of the bur-prepared group decreased only within six months of water storage combined with 12,000 thermocycles. Although the laser irradiation of dentin did not negatively affect the bonding efficiency of different adhesives to sound and caries-affected dentin in the current study, further studies to evaluate the effects of aging procedures on the performance of tested adhesives to irradiated dentin are needed to confirm the current short-term results.

CONCLUSIONS

Within the limitations of this *in vitro* study, the hypotheses tested were accepted. Er,Cr:YSGG laser irradiation did not negatively affect the bonding performance of adhesive systems to sound and caries-affected dentin and the two-step self-etch system produced lower bond strength values than the three-step etch-and-rinse adhesive in sound and caries-affected dentin. Due to an increased interest in the potential for laser technology for hard tissue application, further *in vitro* and *in vivo* investigations of laser-prepared teeth and adhesion are needed.

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