# Influence of Er,Cr:YSGG Laser Treatment on Microtensile Bond Strength of Adhesives to Enamel

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## **Clinical Relevance**

Er,Cr:YSGG laser irradiation produced morphological alterations on enamel, which may adversely influence the bond strength of certain adhesives, depending on their approach and composition.

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DOI: 10.2341/07-124

## **SUMMARY**

The current trend towards minimum-intervention dentistry has introduced laser technology as an alternative technique for cavity preparation. This study assessed the null hypothesis that enamel prepared either by Er,Cr:YSGG laser or conventional diamond bur is equally receptive to adhesive procedures. The buccal and lingual surfaces of 35 sound human molars were prepared with Er,Cr:YSGG laser or a medium-grit diamond bur. One etch&rinse (OptiBond FL) and three self-etch adhesives (Adper Prompt L-Pop, Clearfil SE Bond and Clearfil S³ Bond) were applied on laser-irradiated and bur-cut enamel, followed by the application of a 5-6 mm build-up

of Z100. The micro-tensile bond strength (uTBS) was determined after 24 hours of storage in water at 37°C. Prepared enamel surfaces and failure patterns were evaluated using a stereomicroscope and a field-emission-gun scanning electron microscope (Feg-SEM). The µTBS to laser-irradiated enamel was significantly lower than to bur-cut enamel (p<0.05), with the exception of Clearfil S<sup>3</sup> Bond, which bonded equally effectively to both substrates. The latter presented the highest µTBS on laser-irradiated enamel, though it was not statistically different from the uTBS of OptiBond FL. SEM analysis revealed significant morphological alterations of the laser-irradiated enamel surface, such as areas of melted and recrystalized hydroxyapatite and deep extensive micro-cracks. In conclusion, the bonding effectiveness of adhesives to laser-irradiated enamel depends not only on the structural substrate alterations induced by the laser, but also on the characteristics of the adhesive employed.

### INTRODUCTION

In recent years, the use of laser irradiation for cavity preparation has been widely discussed and many kinds of laser equipment have been employed for this purpose.1 Erbium laser devices, however, have been considered the most efficient and safe systems for cavity preparation, with a noticeable ability to remove dental hard tissues, in addition to providing minimum side effects to the pulp and surrounding tissues.<sup>1-3</sup> Some other advantages are also related to the use of erbium lasers for operative dentistry procedures, such as the absence of vibration, less discomfort to the patient, less risk of cross-contamination, selective caries removal and, in many cases, elimination of local anesthesia.<sup>4</sup> On the other hand, depending on the laser parameters employed, disadvantages of the use of erbium lasers have also been reported, such as potential heating and weakening of the dental hard tissues.<sup>4-5</sup> In light of minimal-invasive dentistry, laser technology has been widespread as an alternative to the conventional use of diamond burs.<sup>5</sup> Its precise ablation of dental hard tissues guarantees the accomplishment of a more conservative cavity design, frequently resulting in preparations made strictly in enamel, such as those for the treatment of pit-and-fissure lesions.4

Nevertheless, the use of laser technology in operative dentistry should not only fulfill the requirements of a conservative treatment, but the laser should also prepare the cavity substrate for the subsequent use of bonding materials.<sup>6</sup> Recently, several investigations have evaluated the chemical and morphological aspects of laser-irradiated primary and permanent enamel and the feasibility of performing conventional adhesive procedures on these substrates.<sup>4-5,7-10</sup>

Micro-explosions during laser ablation are able to eject hard tissue particles from the irradiated substrate, resulting in a typical imbricate patterned surface with an evidently rough aspect without smearlayer production.8 These morphological features seem to be quite favorable for adhesion, especially considering that the interaction of self-etch adhesives with dental substrates is expected to be better in the absence of smear debris. 11 However, the results presented in the literature are still controversial, leading to open-ended discussions concerning the real benefits of laser irradiation in combination with the currently available adhesive systems. 4-5,8-10 These controversies are probably ascribed to variations in irradiation parameter settings employed in the different studies, as well as to differences in erbium-based laser systems (Er:YAG versus Er,Cr:YSGG).12 Although both erbium lasers emit a similar wavelength, which coincides with the major absorption band of water and hydroxyapatite, different laser systems cannot be compared solely based on single and specific parameter settings. Additional parameters, such as frequency, fluence, air/water spray cooling, pulse formation, pulse width, beam profile and outcoupling conditions, should also be taken into account, since they are highly responsible for the thermal effects produced on the target tissue.10,12 Considering several of those parameters, Straßl and others<sup>12</sup> found that dental hard tissues are more thermally affected by an Er:YAG than by an Er, Cr: YSGG laser. Therefore, considering that both chemical and physical alterations of the irradiated tissues are mainly a function of the temperature produced by the laser,13 the Er,Cr:YSGG laser seems promising for cavity preparation and pre-adhesion surface treatment. Nevertheless, only a small number of studies has evaluated the effects of the Er,Cr:YSGG laser on the bonding effectiveness of adhesives to dental substrates. Earlier studies were carried out almost exclusively by using Er:YAG laser Consequently, the purpose of this study was 1) to evaluate the micro-morphological characteristics of Er, Cr: YSGG laser-irradiated enamel and 2) to assess the bonding effectiveness of contemporary adhesives bonded to Er, Cr:YSGG laser-irradiated enamel using microtensile bond strength (µTBS) testing. The null hypothesis tested was that enamel prepared either by laser-irradiation or regular diamond bur is equally receptive to adhesive procedures, regardless of the adhesive system employed.

### **METHODS AND MATERIALS**

Thirty-five non-carious human third molars were stored in 0.5% chloramine solution at 4°C and used within one month after extraction. Thirty-two teeth were employed for  $\mu$ TBS testing, while the remaining three molars were designated for SEM analysis of the laser-irradiated enamel surface. First, the molars

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Adhesive (manufacturer) Classification	Composition (Batch #)	Application
<b>OptiBond FL</b> (Kerr, Orange, CA, USA) <i>Three-step etch&amp;rinse</i>	Etchant: 37.5% phosphoric acid, silica thickener (410643) Primer: HEMA, GPDM, PAMM, ethanol, water, photo initiator (417174) Bond: TEGDMA, UDMA, GPDM, HEMA, Bis-GMA, filler, photo initiator (421941)	Apply the etchant for 15 seconds; rinse for 15 seconds; gently air dry for 5 seconds; scrub the surface for 15 seconds with primer; gently air dry for 5 seconds; apply a thin coat of bonding agent and light cure for 30 seconds.
Clearfil SE Bond (Kuraray, Tokyo, Japan) Two-step self-etch	Primer: 10-MDP, HEMA, hydrophilic dimethacrylate, photo initiator, water (00480A) Bond: 10-MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate, microfiler (00666A)	Apply the primer for 20 seconds; gently air blow; apply the bond and light cure for 10 seconds.
Adper Prompt L-Pop (3M ESPE, Seefeld, Germany) One-step self-etch	Liquid 1: Methacrylated phosphoric esters, Bis-GMA, camphorquinone, stabilizers Liquid 2: Water, HEMA, polyalkenoic acid, stabilizers (199474)	Activate blisters; apply adhesive and scrub the surface for 15 seconds; gently air dry; apply second coat without rubbing; air dry to a thin film and light cure for 10 seconds.
Clearfil S³ Bond (Kuraray, Tokyo, Japan) One-step self-etch	10-MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, photo initiator, ethyl alcohol, water, microfiler (00001A)	Apply adhesive and leave it in place for 20 seconds; dry by blowing high-pressure air for 5 seconds and light cure for 10 seconds

were mounted in gypsum blocks in order to facilitate specimen manipulation. The teeth selected for µTBS testing were randomly divided into eight groups (n=4), determined by the adhesive system (Table 1) and the surface treatment employed (bur-cut or laser-irradiated enamel).

# **Specimen Preparation**

Buccal and lingual enamel were abraded using a highspeed medium-grit (100 µm) diamond bur (842; Komet, Lemgo, Germany) under water cooling using the MicroSpecimen Former (University of Iowa, Iowa City, IA, USA) in order to obtain flat enamel surfaces covered by a standardized smear layer. The absence of dentin on the resultant substrate was controlled by means of a stereomicroscope (Wild M5A, Heerbrugg, Switzerland). For laser-irradiated specimens, similar bur-flattened enamel surfaces were used and prepared with the Er, Cr: YSGG laser device (Waterlase; Biolase Technology, Inc, San Clemente, CA, USA) according to the following parameters: wavelength of 2.78 µm, pulse frequency of 20 Hz, pulse duration of 140 useconds, spot size of 600 µm in diameter, power of 6.0 W, energy density of 107.1 J/cm<sup>2</sup>, air pressure setting of 90% and water pressure setting of 75%. The irradiation was performed in the non-contacted and focused mode, with a cylindrical fiber tip positioned perpendicular to the enamel surface at the distance of 1 mm from the target tissue. Subsequently, the adhesives were applied on the bur-cut or laser-irradiated enamel surfaces strictly following the respective manufacturer's instructions (Table 1). After adhesive procedures, the surfaces were built-up with Z100 (3M ESPE, St Paul, MN, USA) in three to four layers up to a height of 5-6 mm. Each increment was light-cured for 40 seconds using an Optilux 500 light-curing unit (Demetron/Kerr, Danbury, CT, USA) with a light output not less than 550 mW/cm<sup>2</sup>. All the specimens were stored in distilled water at 37°C for 24 hours before µTBS testing.

### **Microtensile Bond Strength Testing**

The specimens were serially sectioned perpendicular to the bonding surface using the Isomet saw under running water in order to obtain rectangular samples about 2x2 mm wide and 8-9 mm long. The specimens were then mounted in the MicroSpecimen Former and trimmed at the biomaterial-tooth interface into an hour-glass shape with a diameter of about 1.1 mm using a cylindrical fine-grit (30 µm) diamond bur (5835KREF, Komet, Lemgo, Germany) in a high speed contra-angle handpiece under air/water coolant. The specimens were then fixed to a Ciucchi's jig with cyanoacrylate glue (Model Repair II Blue; Dentsply-Sankin, Ohtawara, Japan) and stressed until failure at a crosshead speed of 1 mm/minute using a universal testing device (LRX; Lloyd, Hampshire, UK) with a load cell of 100 N (Figure 1). The occurrence of failure prior to the actual testing was included in the calculation of the mean µTBS as 0 MPa, with an explicit note of the number of such pre-testing failures (ptf) in each group. The mode of failure was determined at a magnification of 50x using the low power stereomicroscope Wild M5A and recorded as "adhesive" (interfacial failure), "cohesive in enamel," "cohesive in resin" (including failures either within resin composite or adhesive layer) or "mixed" (including more than one of the previously described patterns).

The obtained data were statistically analyzed using the software GMC8.1 (FORP-USP, Ribeirão Preto, Brazil). The Kruskal-Wallis test and Dwass-Steel-

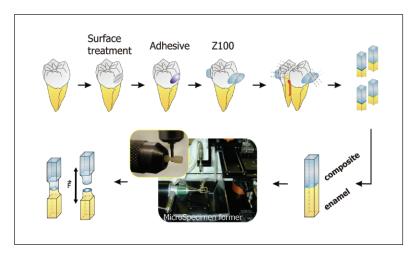


Figure 1. Scheme illustrating the study design.

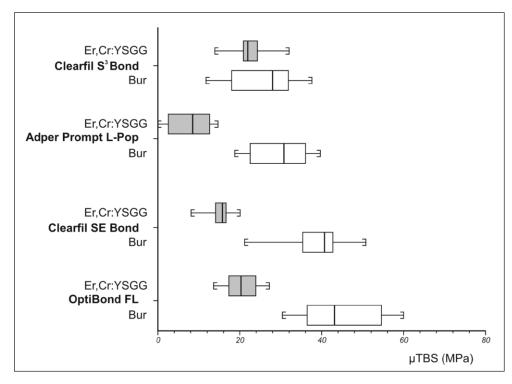


Figure 2. The box plot of enamel micro-tensile bond strength (µTBS) results. The box represents the spreading of the data between the lower and the upper quartile. The central vertical line represents the median. The whiskers extend to the minimum and maximum value.

Table 2: Micro-tensile Bond Strength to Enamel (MPa)					
	OptiBond FL	Clearfil SE Bond	Adper Prompt L-Pop	Clearfil S <sup>3</sup> Bond	
Diamond bur μTBS ± SD ptf/N	45.2 ± 9.6 <sup>A</sup> 0/13	38.8 ± 7.0 <sup>A</sup> 0/23	29.6 ± 6.8 <sup>B</sup> 0/16	25.3 ± 8.3° 0/14	
Er,Cr:YSGG μTBS ± SD ptf/N	20.5 ± 4.1° 0/13	15.1 ± 3.3 <sup>E</sup> 0/12	7.9 ± 5.3 <sup>F</sup> 2/13	22.6 ± 4.6 <sup>c,b</sup> 0/15	

<sup>\*</sup>Data with the same superscript are not statistically significantly different (Kruskal-Wallis test, p<0.05). μTBS: mean micro-tensile bond strength; SD: standard deviation; ptf: pre-testing failure; N: total number of specimens

Chritchlow-Flingner multiple comparisons test were performed to determine statistical differences at a significance level of 5%.

# **Feg-SEM Evaluation**

Following failure analysis performed using a stereomicroscope, two or three representative specimens were processed for field-emission gun scanning electron microscopy (Feg-SEM; Philips XL30, Eindhoven, The Netherlands). Common procedures for SEM specimen preparation were employed, including fixation in 2.5% glutaraldehyde in cacodylate buffer solution, dehydration in ascending concentrations of ethanol and chemical drying using HMDS. <sup>14</sup> The specimens were mounted on aluminum stubs and gold sputter coated using a gold sputtering device

(Sputtering Device 07 120, Balzers Union, Liechtenstein).

Three additional third molars were prepared and laser-irradiated in the same manner as for µTBS testing. The unbonded enamel specimens were evaluated under a stereomicroscope at a magnification of 50x, then processed for Feg-SEM analysis as mentioned above.

# **RESULTS**

The mean µTBS, standard deviation, total number of specimens and number of pre-testing failures are summarized per group in Table 2 and graphically presented in box plots in Figure 2. All the adhesive systems presented a significantly lower µTBS when applied on laser-irradiated enamel, with the exception of Clearfil S³ Bond, which presented no statistically significant difference when applied on bur-cut or

laser-irradiatedsubstrate. To bur-cut enamel. Clearfil S3 Bond showed the lowest μTBS mean when compared to the other bonding systems. Conversely, the

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	Optibond FL	Clearfil SE Bond	Adper Prompt L-Pop	Clearfil S <sup>3</sup> Bond
Diamond bur				
Adhesive	15	17	0	36
Mixed	31	17	75	64
Cohesive in resin	39	61	19	0
Cohesive in enamel	15	5	6	0
Er,Cr:YSGG				
Adhesive	85*	92*	100*	100*
Mixed	15	8	0	0
Cohesive in resin	0	0	0	0
Cohesive in enamel	0	0	0	0

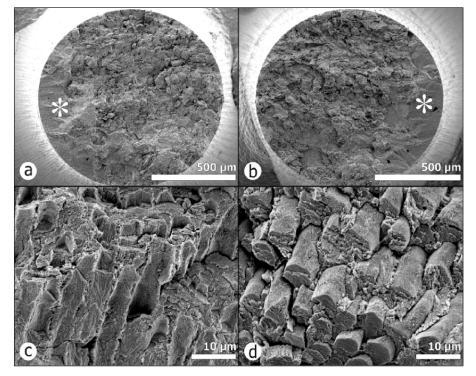


Figure 3. Feg-SEM of a fractured surface of Optibond FL bonded to laser-irradiated enamel after µTBS testing. Photomicrographs show the fractured surface at the enamel side (a) and at the composite side (b). The asterisks point to an area of cohesive failure in the bonding agent. (c) Higher magnification of (a), representing cohesive failure in enamel, a few micrometers below the adhesive interface. This typical failure pattern was observed for all adhesives used in this study when applied to laser-irradiated surfaces. (d) Higher magnification of (b), showing that enamel was cohesively fractured. Observe the presence of microscopically visible enamel fragments still attached to the composite side.

same adhesive presented the highest  $\mu$ TBS when bonded to laser-irradiated enamel (22.6 MPa), though it was not statistically different from the etch&rinse adhesive OptiBond FL (20.5 MPa) but significantly higher than the mild two-step self-etch adhesive Clearfil SE Bond (15.1 MPa) and even much higher than the strong one-step self-etch adhesive Adper Prompt L-Pop (7.9 MPa). The distribution of fracture patterns is presented in Table 3. Stereomicroscope

evaluation determined a higher incidence of adhesive failures for laser-irradiated specimens. However, presence of small, not macroscopically visible enamel fragments was observed at the composite side of the fractured

specimens when they were analyzed using Feg-SEM (Figure 3). The occurrence of adhesive failures revealed different patterns of adhesive/substrate interaction, depending on the cavity-preparation technique. For bur-cut enamel, the prisms were exposed transversally (Figure 4), while they were primarily displayed longitudinally when the enamel surface was laser irradiated (Figure 3).

When analyzed by means of the stereomicroscope, the laser-irradiated surface showed an irregular surface topography and an opaque white appearance, with no evidence of carbonization (brown spots). Under Feg-SEM, ablated enamel revealed an irregular surface, with the presence of well-defined cracks and enamel prisms exposed longitudinally. Zones of melting and recrystallization were also observed (Figure 5).

# **DISCUSSION**

The use of erbium laser technology has been widely studied in operative dentistry since initially and experimentally being employed by Keller and Hibst.<sup>2</sup> By varying some parameters, laser irradiation can be used for

several applications, such as caries removal, <sup>15</sup> conditioning of dental hard tissues, <sup>10</sup> increasing tooth acid resistance <sup>16</sup> and cavity preparation. <sup>9</sup> In the current study, the authors evaluated the µTBS of different adhesives to laser-irradiated enamel using the parameters recommended by the manufacturer for cavity preparation. Therefore, the maximum output power was used, providing increased values of energy density and, as a consequence, a higher efficiency on laser

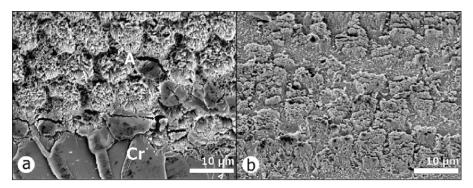


Figure 4. Feg-SEM of a fractured surface of Adper Prompt L-Pop bonded to bur-cut enamel after µTBS testing. (a) Photomicrograph of the fractured surface at the enamel side, presenting a typical mixed fracture that included areas of both adhesive (A) and cohesive failure within the adhesive layer (Cr). The prisms were exposed transversally and a typical Type 1 etching pattern can be noticed. (b) Photomicrograph of the fractured surface at the composite side, showing an area of adhesive failure.

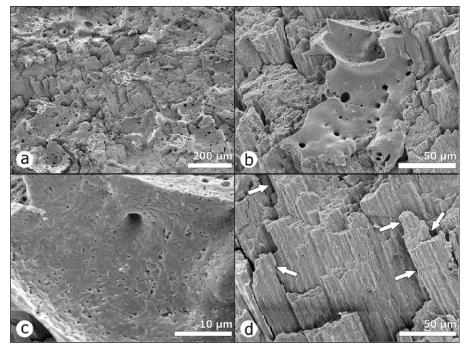


Figure 5. Feg-SEM photomicrographs of Er,Cr:YSGG-irradiated enamel surface: (a) typical morphology of laser-ablated enamel, showing an irregular topography; (b) well-defined zone of melted enamel surrounded by laser-ablated enamel; (c) higher magnification of (b), revealing the micromorphology of melted and resolidified enamel after laser irradiation. A crystalline pattern is clearly visible; (d) extensive micro-cracks and prominent enamel particles partially attached to the remaining surface are observed (arrows).

ablation.<sup>7</sup> However, the use of higher values of energy density has also been related to a more harmful thermal effect on dental hard tissues, <sup>15,17</sup> particularly when laser-irradiation is not accompanied by a continuous water spray.<sup>7</sup> Being considered a laser-powered hydrokinetic system, the Er,Cr:YSGG laser source delivers photons into an air-water spray matrix directed straight to the target tissue. Thus, during irradiation, the molecules of water on the dental surface absorb the incident radiation, causing sudden heating and water

evaporation. As a result, a high-stream pressure occurs, inducing a violent, yet controlled micro-expansion and the ejection of dental hard tissues.3,7 Therefore, water plays an important role during Er, Cr:YSGG irradiation not only by suppressing the temperature, but also by increasing the cutting efficiency, since the ablation mechanism is water dependent.7 However, zones of melting and recrystallization were clearly observed in the current study, despite the use of constant water cooling during laser-irradiation (Figure 5). Although former studies have reported minimal thermal effects when irradiation is performed under adequate water cooling,7,17 it should be considered that a higher energy density was used in the current study.

The morphological analysis showed that laser-irradiated enamel displayed longitudinal exposure of its rods as a consequence of the micro-explosions induced by laser irradiation. During the occurrence of laser ablation, the prisms are probably separated from one another within the interprismatic substance, which represents the weakest path for crack propagation.18 The presence of micro-cracks throughout the irradiated area has also been reported in the literature as a consequence of laser ablation, 4,7,16,19 in accordance with the findings of the current study. Due to the micro-explosions induced by laser-irradiation, some particles of enamel are completely ejected from the dental surface, while certain others seem to remain partially attached to the underlying substrate. The micro-cracks observed in this study were located under such affected, but not released particles (Figure 5d), resulting in weakening of the substrate. In addition to morphological alterations, it is necessary to consider

the chemical changes produced by erbium lasers in irradiated tissues. Higher quantities of Ca and P are available in the irradiated dental surface<sup>17</sup> inasmuch as the organic components evaporate due to the increased temperature in the irradiated area. <sup>17,20</sup>

The irregularities produced by laser irradiation at the enamel surface suggest an imbricate pattern, which seems to be quite favorable for adhesion. Moreover, the absence of a smear layer is also advantageous, espe454 Operative Dentistry

cially considering the so-called "mild" self-etch adhesives. It has been reported that differences in the quality and quantity of the smear layer can affect the ability of such adhesives to properly demineralize and penetrate the underlying substrate. 11 However, according to the results obtained in the current study, OptiBond FL, Clearfil SE and Adper Prompt L-Pop bonded more effectively to bur-cut than to laser-irradiated enamel, by which the null hypothesis was rejected. In agreement with the results of the current study, Dunn and others9 also reported an adverse influence of laser irradiation on adhesion to enamel when an Er:YAG laser was used with parameters for cavity preparation. Proper additional comparisons are unavailable due to a lack of studies assessing the bond strength of adhesives to Er,Cr:YSGG-laser irradiated enamel using energy densities for cavity preparation.

It can be supposed that the lower µTBS provided by laser-irradiated enamel is partially related to the morphological changes produced on its surface after ablation. As mentioned above, the presence of micro-cracks under partially attached enamel particles may provide a weakened substrate, which is more prone to the occurrence of fractures during bond strength testing. This hypothesis probably explains the high incidence of enamel fragments still attached to the resin side of the specimen after uTBS testing, as previously reported by De Munck and others.8 Actually, it should be emphasized that the presence of micro-cracks on enamel can set a limit on the uTBS results, whereas the obtained data can be better related to the ultimate strength of the affected substrate than to the bonding effectiveness of the adhesive. Furthermore, the occurrence of thermal alterations on the irradiated enamel, such as melting and chemical changes, can also render the enamel less receptive for adhesion. It has been reported that Er, Cr:YSGG-laser irradiation can markedly reduce the acid dissolution of dental hard tissues, playing an important role in the prevention of secondary caries.<sup>17</sup> However, this acid resistance may hamper the acid conditioning of enamel during adhesive procedures, especially considering the limited etching potential of "mild" self-etch systems.21

The thick, clinically relevant smear layer produced by the diamond bur seems to have hindered the bonding effectiveness of Clearfil S³ Bond, which presented the lowest µTBS to bur-cut enamel. While "strong" self-etch systems, such as Adper Prompt L-Pop, are effective in dissolving the smear layer and demineralizing the underlying substrate, Clearfil S³ Bond, which is considered an "ultra-mild" self-etch adhesive (pH≈2.7), is less efficient for this purpose. It has been stated that the acidic potential of mild self-etch adhesives can be buffered by the mineral contents of the smear layer, preventing a proper interaction between the adhesive and substrate.<sup>22</sup> On the other hand, along with

OptiBond FL, Clearfil S³ Bond obtained the highest bonding effectiveness when applied on laser-irradiated enamel. In addition to presenting a smear layer-free surface, laser-irradiated enamel shows a significant increase in calcium in its composition,¹¹ which seems to play an important role in the bonding effectiveness of Clearfil S³ Bond, a 10-MDP-based adhesive. The molecular structure of 10-MDP provides the bonding agent with the ability to partially decalcify dental hard tissues, penetrate the demineralized substrate and chemically bond to the remaining calcium.²¹

Clearfil SE Bond is also a 10-MDP-based adhesive produced by the same manufacturer as Clearfil S<sup>3</sup> Bond. However, it is still unclear why Clearfil SE Bond presented a lower uTBS to laser-irradiated enamel when compared to Clearfil S<sup>3</sup> Bond (Table 2). The main difference between both adhesives is that Clearfil S<sup>3</sup> Bond is applied in a single step, while Clearfil SE Bond requires a prior priming procedure. However, a separate step for priming the laser-irradiated enamel seems unnecessary; whereas both smear-layer removal and surface roughening are provided by laser ablation, resulting in a high surface energy.<sup>10</sup> Moreover, some components of the primer, such as water and HEMA, may still remain on the enamel surface after application.23 Those remnants may hinder the penetration of some important adhesive components, such as MDP, Bis-GMA and dimethacrylates, which are strongly responsible for reinforcement of the substrate. Since Clearfill S<sup>3</sup> Bond does not require a previous priming step, it seems to be more effective in penetrating and sealing the micro-cracks induced by laser irradiation, thus providing reinforcement of such an affected substrate. This hypothesis may explain the higher bond strengths achieved by Clearfil S3 Bond on laser-irradiated enamel, as observed in Table 2.

There are several benefits to using dental lasers, but each application requires a precise definition of the parameters in regard to the properties of the target tissue, the characteristics of the laser device and the interaction type. In addition to determining less harmful parameters for cavity preparation, future research should focus on the development of new adhesive systems in order to provide favorable interaction with the laser-modified enamel surface. The use of an ultra-mild one-step self-etch adhesive based on a chemical bonding approach seems to be a promising option for laser-irradiated enamel surfaces

# CONCLUSIONS

Taking into account the parameters employed in this study, laser irradiation produced morphological alterations on enamel, which adversely influenced the bond strength of the adhesives tested. As an exception, the bonding effectiveness of Clearfil S<sup>3</sup> Bond to enamel was not influenced by laser irradiation.

Therefore, it is possible to conclude that the bonding interaction of adhesives to enamel depends not only on the morphological aspects of the substrate, but also on the characteristics of the adhesive employed.

(Received 24 July 2007)

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