

Original Article

Effect of Erbium:yttrium Aluminum Garnet Laser on Bond Strength of a Total-etch Adhesive System to Caries-affected Dentin on Gingival Wall

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ABSTRACT

Purpose: To assess the effect of the erbium:yttrium aluminum garnet (Er:YAG) laser on bond strength of a total-etch adhesive system to the caries-affected dentin on the gingival wall. **Materials and Methods:** Ten human molars with proximal carious lesions were randomly divided into two groups. In the first group, the carious dentin was removed with a bur, whereas in the second group it was removed with the Er:YAG laser. Carious lesions were excavated with one of these two techniques until laser fluorescence values decreased to 15 in the center of the lesions. The teeth were then restored with a total-etch adhesive system (Adper Single Bond 2) and composite resin (Filtek Z250). Five teeth from each group were sectioned to obtain 1 mm² stick-shaped microtensile specimens from each tooth. Twenty-five specimens were obtained for each group with using this technique. The data were analyzed in independent-samples *t*-test ($\alpha = 0.05$). For each removal technique, one sample was analyzed using scanning electron microscopy. **Results:** No statistically significant differences were found between the bond strength of the Er:YAG laser and the bur-treated groups ($P > 0.05$). **Conclusion:** The Er:YAG laser treatment did not negatively affect the bonding performance of the total-etch adhesive system to caries-affected dentin on the gingival wall.

KEYWORDS: Bond strength, bur, caries removing, dentin, erbium:yttrium aluminum garnet laser, gingival wall

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INTRODUCTION

Dental caries is an infectious disease that can lead to pain, tooth loss, infection, and in severe cases, pulp death. Despite its reduction in many countries, dental caries is still one of the most common diseases throughout the world.^[1]

Bonding with adhesive system to enamel is highly predictable, but bonding to dentin is less predictable, especially when the bonding is to the gingival cavity wall of Class II posterior resin-based composite preparations.^[2] Poor marginal adaptation and considerable leakage have been shown *in vitro* in cavities with the cervical margin located at or below the cervicoenamel junction.^[3,4] Furthermore, the dentinal tubule orientation of approximal cavities on the gingival wall is different from that of those on the pulpal wall.^[5-7] A previous study that used a water-based adhesive in Class II preparations

found that the bond to the gingival wall was weaker than the bond to the axial wall.^[8]

Cariou dentin consists of two layers

The two layers that comprise carious dentin are an outer necrotic, highly infected layer, and an inner, less infected and demineralized layer. Although demineralized, the inner layer, which is characterized by the presence of acid-resistant and water infiltration-hampered calcium phosphate crystals in the dentinal tubules, is potentially repairable through dental restorations.^[9]

The treatment of carious lesions today is accompanied by the removal of affected hard tissues. A commonly

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used method for restorative procedures is to use rotary instrumentation with burs at low and high speeds. In addition to some of the advantages of this technique, such as speed and low cost, however, it can cause patients discomfort and thus require local anesthesia.^[1] These disadvantages have led to the development of new technologies for dental hard tissue preparation and caries removal such as laser irradiation.^[10]

The use of laser technology as an alternative to traditional mechanical rotating instruments for cavity preparation has been introduced. Various types of laser, such as the carbon dioxide laser (CO₂ laser, the neodymium:yttrium aluminum garnet (Nd:YAG) laser, the erbium:yttrium aluminum garnet (Er:YAG) laser, the erbium chromium:yttrium scandium gallium garnet (Er,Cr:YSGG) laser, have been introduced into dental clinics.^[11,12]

Of these, the Er:YAG laser has proved to be particularly advantageous. Its 2940 nm beam wavelength is close to the maximum absorption of water, which means that incoming laser light is totally absorbed by water present in the tissue. The concentrated release of energy in the tissue leads to the explosion-like vaporization of water, with teeth fragments catapulted out of the hard substance.^[13] Under a water spray, this laser is able to prepare cavities successfully in enamel and dentin without damaging dental pulp tissue.^[13,14] This equipment also presents the advantage of being more comfortable for the patient and in many cases, can eliminate the necessity of anesthesia.^[15-17]

The bonding of an adhesive to dentin is complex, and bond strength is one of the most important performance parameters of dental adhesives.^[18] It has been claimed that bond strength depends on both the type of bonding surface and the adhesive used.^[19] In clinical situations, the bonding surface most frequently is caries-affected dentin. Previous studies have shown that bond strength to normal dentin with total-etch and self-etch adhesives is significantly higher than to caries-affected dentin.^[20,21]

The aim of this study was to compare the microtensile strengths of composite bonded to caries-affected human dentin using a total-etch adhesive system after the use of two different techniques to remove the caries: Conventional bur and Er:YAG laser.

The null hypothesis to be investigated in this study was as follows: There are no differences among the bonding values of two different caries removal techniques.

MATERIALS AND METHODS

The study protocol was reviewed and approved by the Ethics Committee of the University of Selcuk, Turkey; the protocol number is 2010/03.

Sampling

The power analysis was established by G*Power version 3.1.9.2 software (Franz Faul, Kiel University, Kiel, Germany). Based on the 1:1 ratio between groups, a sample size of 25 teeth per group would give more than 80% power to detect significant differences with a 0.58 effect size at the 0.05 significance level.

Ten extracted permanent human molars with approximal dentin caries were used to microtensile bond strength (μ TBS) test. The teeth had only mesial or distal approximal caries. All teeth were stored at 4°C in physiologic saline for no longer than 4 weeks after extraction. Any soft tissue was removed and the teeth underwent ultrasonication to remove plaque and other pit and fissure debris. Any teeth showing signs of extraction damage or extensive cavitated lesions with pulpal involvement were discarded from the study. Enamel and superficial dentin of the crown were flattened perpendicular to the long axis of the tooth with a bur until the lesions showed laser fluorescent values of approximately 40–50 (Diagnodent, Kavo Dental, Biberach, Germany). After this, the specimens were washed with de-ionized water for 1 min.^[22]

Experimental groups

The teeth were randomly divided into two groups based on different caries removal techniques (bur and laser).

In the bur removal groups, dentinal caries was removed with a round steel bur (No. 14–16, ISO: 310204001001 021, GebrLemgo, Germany) in a water-cooled, slow-speed handpiece (Bien Air SN 09B0600, Bien, Switzerland).

In the laser removal groups, an Er:YAG laser system (Fidelis Plus III, Fotona Ljubljana, Slovenia) with a laser wavelength of 2.94 μ m was used to remove caries. The power output was 3.5 W, the pulse duration was 300 μ s short pulse mode, and the pulse repetition rate was 10 Hz. Irradiation of a focused beam was performed from a 1 mm distance (energy density: 44 J/cm²). Cylindrical quartz with a diameter of 1 mm (65,320, Fidelis Plus III, Fotona) was mounted to the R14 handpiece for dentin ablation. The irradiated area was continuously cooled using an air and water spray system.

Cariou lesion removal was repeated for each technique until the laser fluorescence (LF) value decreased to approximately 11–20 in the lesion center.^[23] Adhesive (Adper Single Bond 2) was applied according to the manufacturers' instructions. Following the application of the adhesives, the caries-affected region was filled with composite resin (Filtek Z 250, 3M ESPE dental products, Saint Paul, USA). Composite restorations were made incrementally with 1.5 mm layers to a

height of 4–5 mm.^[24] Each layer was photo-cured for 20 s.

The adhesive systems used in the present study, including the manufacturers' instructions, batch numbers, compositions, and application modes, are shown in Table 1.

Microtensile test

A microtensile bond test was used, which is a method that enables the use of multiple specimens of the same tooth. Five teeth from each group were used to μ TBS test. After immersion in water at 37°C for 24 h, approximal sites of the restored teeth were vertically sectioned both mesial-distally, buccal-lingually along their long axis and perpendicular to the gingival wall with a slow-speed diamond saw (Isomet 1000, Buehler Ltd. Lake Bluff, IL, USA) to obtain five 1 mm² stick-shaped microtensile specimens from each tooth [Figure 1]. Each stick was carefully examined in a dissecting microscope ($\times 20$) to ensure that the test site was homogeneous with regard to caries-affected dentin. The thicknesses of the specimens were measured using digital calipers (Mitutoyo, Tokyo, Japan). Twenty-two specimens were obtained for each group with using this technique. All specimens were fixed with cyanoacrylate glue (Zapit; DAVA, Corona, CA, USA) to two surfaces on a linear actuator-driven, offset microtensile testing device (BISCO; Schaumburg, IL, USA), and stressed at a crosshead speed of 1 mm/min until failure. The μ TBS was expressed in MPa and derived by dividing the imposed force (N) at the time of fracture by the bond area (mm²).^[25] The pretesting failures were considered as 0 MPa.

Evaluation using scanning electron microscopy

The aim of scanning electron microscopy (SEM) analysis was to observe the micromorphology of the caries-affected dentin after the use of different caries removal techniques (conventional bur, Er:YAG laser). Two molar teeth with dentinal caries were used in micromorphology evaluation using SEM (JEOL JSM-6390 LV, JEOL Ltd., Tokyo, Japan). The caries removal procedures were carried out the same as for the μ TBS testing, and then

the tooth substrates were fixed in 2.5% glutaraldehyde in a 0.1 M phosphate-buffered solution for 24 h at room temperature. The specimens were dehydrated with increasing ethanol concentrations and submitted to chemical drying in hexamethyldisilazane. After drying at room temperature (24°C), the specimens were gold sputter-coated, and the caries-affected dentin surfaces were observed by SEM. Entire surfaces were scanned, and the most representative areas were photographed at $\times 2000$ magnification.

Statistical analysis

The data were entered into a spreadsheet (Excel; version 4.0, Microsoft, Seattle, WA, USA) for the calculation of descriptive statistics. The data were analyzed independent-samples *t*-test ($\alpha = 0.05$) using the SPSS 13 (SPSS Inc., Chicago, IL, USA) statistical program software for Windows.

RESULTS

Microtensile bond strength

No sample exhibited pre-testing failure. According to the independent-samples' *t*-test results, there were no statistically significant differences found between the μ TBS of the Er:YAG laser and bur-cleaned groups ($P > 0.05$). Mean μ TBS values and standard deviations for the experimental groups are shown in Table 2.

Scanning electron microscopy

For morphological illustration, samples of caries-affected dentin were prepared for SEM. A representative micrograph of a bur group sample is shown in Figure 2, and a laser group sample is shown in Figure 3.

Table 2: The microtensile bond strength values (MPa) (mean \pm standard deviation)

Caries removal techniques	Mean \pm SD
Bur	18.75 \pm 5.95
Er:YAG laser	16.96 \pm 5.04

No statistically significant difference ($P > 0.05$) was found between the groups. Er:YAG=Erbium:yttrium aluminum garnet; SD=Standard deviation

Table 1: Main components and application mode of materials used in the experimental study

Materials	Batch number	Composition	Manufacturer	Application mode
Filtek Z 250	9jx	Bis-GMA, TEGDMA, UDMA, Bis-EMA, zirconia, silica	3M Dental Products, MN, USA	Place increments <2.5 mm and light cure each increment for 20 s
Adper Single Bond 2	Etchant: N225999 Bond liquid: N244468	35% phosphoric acid Bis-GMA, HEMA, polyalkenoic co-polymer ethanol, purified water	3M Dental Products, MN, USA	Etch substrate for 15 s, rinse with water spray and dry gently Apply bonding resin, air-thin, light cure for 10 s

Bis-GMA=Bisphenyl-glycidyl-methacrylate; HEMA=2-hydroxyethyl methacrylate; UDMA=Urethane dimethacrylate; Bis-EMA=Ethoxylated bisphenol A dimethacrylate; TEGDMA=Triethylene glycol dimethacrylate

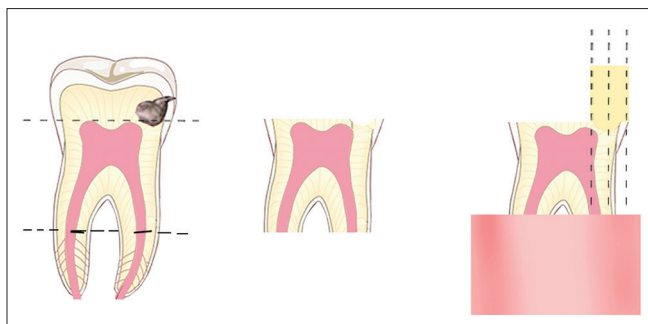


Figure 1: Schematic representation of the samples preparation

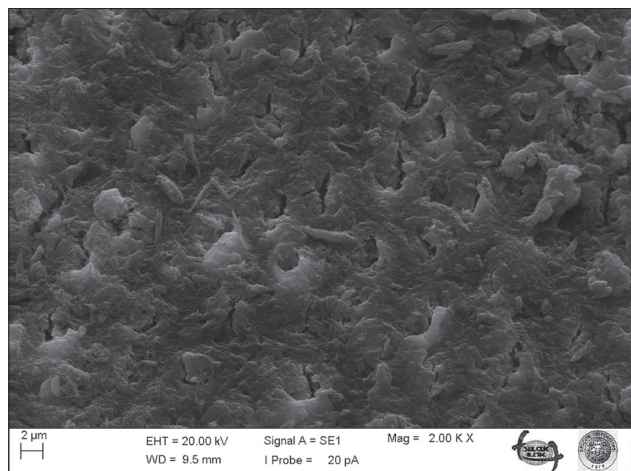


Figure 2: Scanning electron micrograph illustrating the overall morphological aspect of the caries-affected dentine for the bur removal techniques (original $\times 2000$)

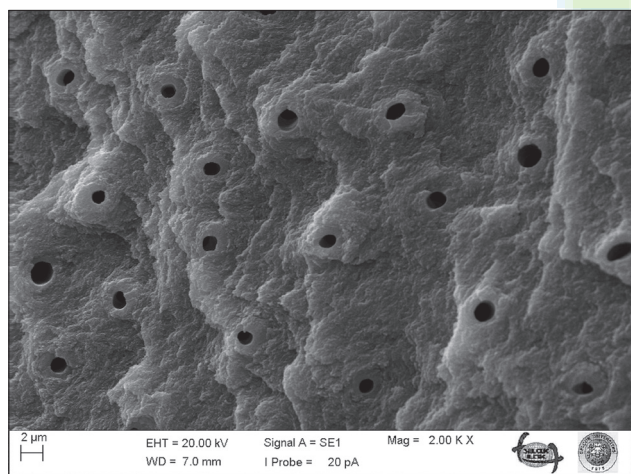


Figure 3: Scanning electron micrograph illustrating the overall morphological aspect of the caries-affected dentine for the dentine ablated by Er:yttrium aluminum garnet laser (original $\times 2000$)

After removing the caries with burs [Figure 2], dentin was covered by a smear layer, completely masking the dentinal tubules (original magnification $\times 2000$).

The dentin treated by the Er:YAG laser [Figure 3] presented opened dentinal tubules distributed on a scaly

surface free of a smear layer, with intertubular dentin more ablated than the peritubular dentin. The surface was generally free of the smear layer, accompanied by open dentinal tubules and irregular and microretentive morphological patterns (original magnification $\times 2000$).

DISCUSSION

This study measured the bond strength of a total-etch dentin adhesive to caries-affected dentin on the gingival wall after the application of two different caries removal techniques. The results of this study support the hypothesis that there are no differences among the bonding values of two different caries removal techniques.

Bond strengths of adhesive system to dental tissues are generally tested in tension or in shear. Many *in vitro* bond strength tests are conducted on flat ground and noncarious dentin surfaces. Although the results of these tests are very useful in terms of comparing the effectiveness of adhesive systems or performing a screening test for experimental bonding systems, flat-ground normal dentin is not the substrate most regularly encountered in clinical situations. Clinicians must usually deal with caries-affected dentin in various locations on a three-dimensional cavity wall.^[5] Bonding to normal dentin with different adhesives has shown bond strengths significantly higher than those to caries-affected dentin. Furthermore, the orientation of dentinal tubules differs at the occlusal and gingival walls of the cavity, which affects the bond strength of adhesives.^[7] Therefore, in this study, the bond strength of a total-etch adhesive on the gingival walls of approximal caries lesions was investigated.

Clinicians use different methods to excavate lesions and remove infected dentin based on pain, color, tactile hardness, dye staining, self-limiting burs, chemical agents, and lasers.^[26-28] Tactile hardness is one of the most common criteria used by clinicians when removing dentin caries. However, it may not be a reliable guide for the clinical removal of caries.^[29] Dye staining is another method to remove carious dentin,^[30] but it can cause the excessive removal of caries-affected or sound dentin.^[31] A method used for residual caries diagnosis is LF.^[32-34] The principle behind the use of this method is that the LF emitted from carious surfaces will be greater than that emitted from sound surfaces.^[35] LF has exhibited greater sensitivity than caries-detecting dyes in caries detection.^[36] Therefore, in this study, LF was used to evaluate residual caries.

μ TBS testing allows for measuring small areas, making it possible to assess the adhesion strength of resin composite to clinically relevant dentin, such as

caries-affected dentin, with specimens of limited size and irregular shape.^[37] This technique permits multiple samples to be prepared from each specimen and allows bonds to be tested after they have been created under clinically relevant conditions without the need for surfaces to be excessively flattened.^[38] The technique eliminates most of the cohesive resin or dentin fractures due to nonuniform stress distributions that are common in more traditional tensile strength test procedures.^[37]

The various techniques such as bur, laser or chemo-mechanical removal are still discussed to remove caries. These techniques create dentin surfaces with different morphology and bonding characteristics.^[39] The removal of dental hard tissues by laser systems is an effective alternative to conventional techniques because they create irregular and retentive micromorphological structures without causing any damage.^[40] After the conventional preparation of a cavity with a bur, an amorphous smear layer including organic and inorganic debris that occludes the tubules is formed on the surface of dentin.^[41] The presence of the 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.^[42] Dentinal surfaces treated with the Er:YAG laser have significantly different characteristics from those treated with conventional bur instruments. Previous studies have shown surfaces treated with bur and covered with a smear layer and dentinal tubules orifices to be plugged with material.^[39,43] The Er:YAG laser-irradiated dentin displayed rough and clean areas without debris accompanied by the exposed orifices of the dentinal tubules, 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.^[39,43]

In the present study, SEM images reveal that, after Er:YAG laser treatment, the surface was generally free of a smear layer [Figure 3]. However, after bur treatment, the dentin was covered by a smear layer that masked the dentinal tubules [Figure 2]. The results of bond strength to caries-affected dentin from the groups treated with the Er:YAG laser were similar to those of the bur-cut group. This was probably due to the use of phosphoric acid on the dentin to remove the smear layer, which partially dissolves the surrounding peritubular dentin, allowing more resin to infiltrate into the dentin tubule.

While some studies have been conducted on caries-affected occlusal dentin surfaces, there is currently no data available in the literature on the μ TBS

of adhesive systems to laser irradiated caries-affected dentin on the gingival wall. The results of the current study agree with data from recent studies by Sattabanasuk *et al.* and Sirin Karaarslan *et al.*^[39,44] The results show that, as for the Er:YAG laser and total-etch, there are no significant differences between the μ TBS of resin and caries-affected dentin compared to the bur treatment. In addition, the above authors also reported that total-etch adhesive systems show higher bond strength than self-etch adhesives to Er laser-irradiated dentin surfaces.^[39,44] Previous studies have shown morphological alterations produced by laser irradiation.^[45] Such alterations can lead to a dentine surface becoming more resistant to demineralization, thus impairing the action of a mild pH primer.^[1] To compensate for the negative effect of Er lasers on adhesion to dentin, some researchers have proposed the application of acid-etching after adhesive procedures with laser irradiation.^[46]

It must be noted that only one test (μ TBS test) was used to evaluate the performance of a total-etch adhesive system. The μ TBS tests are a useful tool to assess the bonding properties between different materials used in restorative dentistry, but no direct extrapolations can be made considering the behavior of these materials under clinical conditions. This may be considered one of the limitations of the current study.

CONCLUSION

Within the limitations of this *in vitro* study, it was found that the Er:YAG laser treatment did not negatively affect the bonding performance of the total-etch adhesive system to caries-affected dentin on the gingival wall. Further *in vitro* and *in vivo* investigations of laser-prepared teeth and adhesives are needed.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Tachibana A, Marques MM, Soler JM, Matos AB. Erbium, chromium:yttrium scandium gallium garnet laser for caries removal: Influence on bonding of a self-etching adhesive system. *Lasers Med Sci* 2008;23:435-41.
2. Purk JH, Healy M, Dusevich V, Glaros A, Eick JD. *In vitro* microtensile bond strength of four adhesives tested at the gingival and pulpal walls of Class II restorations. *J Am Dent Assoc* 2006;137:1414-8.
3. Dietschi D, De Siebenthal G, Neveu-Rosenstand L, Holz J. Influence of the restorative technique and new adhesives on the dentin marginal seal and adaptation of resin composite Class II restorations: An *in vitro* evaluation. *Quintessence Int*

- 1995;26:717-27.
4. Cenci M, Demarco F, de Carvalho R. Class II composite resin restorations with two polymerization techniques: Relationship between microtensile bond strength and marginal leakage. *J Dent* 2005;33:603-10.
 5. Ozer F, Unlü N, Sengun A. Influence of dentinal regions on bond strengths of different adhesive systems. *J Oral Rehabil* 2003;30:659-63.
 6. Bedran-de-Castro AK, Pereira PN, Thompson JY. Influence of load cycling and tubule orientation on ultimate tensile strength of dentin. *J Adhes Dent* 2004;6:191-4.
 7. Cehreli ZC, Akça T. Effect of dentinal tubule orientation on the microtensile bond strength to primary dentin. *J Dent Child (Chic)* 2003;70:139-44.
 8. Purk JH, Dusevich V, Glaros A, Spencer P, Eick JD. *In vivo* versus *in vitro* microtensile bond strength of axial versus gingival cavity preparation walls in Class II resin-based composite restorations. *J Am Dent Assoc* 2004;135:185-93.
 9. Fusayama T. Two layers of carious dentin; diagnosis and treatment. *Oper Dent* 1979;4:63-70.
 10. Ergücü Z, Celik EU, Unlü N, Türkün M, Ozer F. Effect of Er, Cr:YSGG laser on the microtensile bond strength of two different adhesives to the sound and caries-affected dentin. *Oper Dent* 2009;34:460-6.
 11. Wigdor H, Abt E, Ashrafi S, Walsh JT Jr. The effect of lasers on dental hard tissues. *J Am Dent Assoc* 1993;124:65-70.
 12. Wigdor HA, Walsh JT Jr., Featherstone JD, Visuri SR, Fried D, Waldvogel JL. Lasers in dentistry. *Lasers Surg Med* 1995;16:103-33.
 13. Mehl A, Kremers L, Salzmann K, Hickel R. 3D volume-ablation rate and thermal side effects with the Er:YAG and Nd:YAG laser. *Dent Mater* 1997;13:246-51.
 14. Contente MM, de Lima FA, Galo R, Pécora JD, Bachmann L, Palma-Dibb RG, *et al.* Temperature rise during Er:YAG cavity preparation of primary enamel. *Lasers Med Sci* 2012;27:1-5.
 15. Takamori K, Furukawa H, Morikawa Y, Katayama T, Watanabe S. Basic study on vibrations during tooth preparations caused by high-speed drilling and Er:YAG laser irradiation. *Lasers Surg Med* 2003;32:25-31.
 16. Chaiyavej S, Yamamoto H, Takeda A, Suda H. Response of feline intradental nerve fibers to tooth cutting by Er:YAG laser. *Lasers Surg Med* 2000;27:341-9.
 17. Parker S. Laser regulation and safety in general dental practice. *Br Dent J* 2007;202:523-32.
 18. Nakajima M, Sano H, Burrow MF, Tagami J, Yoshiyama M, Ebisu S, *et al.* Tensile bond strength and SEM evaluation of caries-affected dentin using dentin adhesives. *J Dent Res* 1995;74:1679-88.
 19. Toledano M, Osorio R, Ceballos L, Fuentes MV, Fernandes CA, Tay FR, *et al.* Microtensile bond strength of several adhesive systems to different dentin depths. *Am J Dent* 2003;16:292-8.
 20. Nakajima M, Sano H, Urabe I, Tagami J, Pashley DH. Bond strengths of single-bottle dentin adhesives to caries-affected dentin. *Oper Dent* 2000;25:2-10.
 21. Yoshiyama M, Tay FR, Doi J, Nishitani Y, Yamada T, Itou K, *et al.* Bonding of self-etch and total-etch adhesives to carious dentin. *J Dent Res* 2002;81:556-60.
 22. Cehreli ZC, Yazici AR, Akca T, Ozgünlaltay G. A morphological and micro-tensile bond strength evaluation of a single-bottle adhesive to caries-affected human dentine after four different caries removal techniques. *J Dent* 2003;31:429-35.
 23. Yonemoto K, Eguro T, Maeda T, Tanaka H. Application of DIAGNOdent as a guide for removing carious dentin with Er:YAG laser. *J Dent* 2006;34:269-76.
 24. Sano H, Shono T, Sonoda H, Takatsu T, Ciucchi B, Carvalho R, *et al.* Relationship between surface area for adhesion and tensile bond strength – Evaluation of a micro-tensile bond test. *Dent Mater* 1994;10:236-40.
 25. Banerjee A, Kellow S, Mannocci F, Cook RJ, Watson TF. An *in vitro* evaluation of microtensile bond strengths of two adhesive bonding agents to residual dentine after caries removal using three excavation techniques. *J Dent* 2010;38:480-9.
 26. Itoh K, Kusunoki M, Oikawa M, Tani C, Hisamitsu H. *In vitro* comparison of three caries dyes. *Am J Dent* 2009;22:195-9.
 27. Pugach MK, Strother J, Darling CL, Fried D, Gansky SA, Marshall SJ, *et al.* Dentin caries zones: Mineral, structure, and properties. *J Dent Res* 2009;88:71-6.
 28. Neves Ade A, Coutinho E, Cardoso MV, de Munck J, Van Meerbeek B. Micro-tensile bond strength and interfacial characterization of an adhesive bonded to dentin prepared by contemporary caries-excitation techniques. *Dent Mater* 2011;27:552-62.
 29. Fusayama T, Okuse K, Hosoda H. Relationship between hardness, discoloration, and microbial invasion in carious dentin. *J Dent Res* 1966;45:1033-46.
 30. Hosoya Y, Taguchi T, Arita S, Tay FR. Clinical evaluation of polypropylene glycol-based caries detecting dyes for primary and permanent carious dentin. *J Dent* 2008;36:1041-7.
 31. Hosoya Y, Taguchi T, Tay FR. Evaluation of a new caries detecting dye for primary and permanent carious dentin. *J Dent* 2007;35:137-43.
 32. Shi XQ, Welander U, Angmar-Månsson B. Occlusal caries detection with KaVo DIAGNOdent and radiography: An *in vitro* comparison. *Caries Res* 2000;34:151-8.
 33. Shi XQ, Tranaeus S, Angmar-Månsson B. Validation of DIAGNOdent for quantification of smooth-surface caries: An *in vitro* study. *Acta Odontol Scand* 2001;59:74-8.
 34. Lussi A, Megert B, Longbottom C, Reich E, Francescut P. Clinical performance of a laser fluorescence device for detection of occlusal caries lesions. *Eur J Oral Sci* 2001;109:14-9.
 35. Sundström F, Fredriksson K, Montán S, Hafström-Björkman U, Ström J. Laser-induced fluorescence from sound and carious tooth substance: Spectroscopic studies. *Swed Dent J* 1985;9:71-80.
 36. Yazici AR, Baseren M, Gokalp S. The *in vitro* performance of laser fluorescence and caries-detector dye for detecting residual carious dentin during tooth preparation. *Quintessence Int* 2005;36:417-22.
 37. Shono Y, Ogawa T, Terashita M, Carvalho RM, Pashley EL, Pashley DH. Regional measurement of resin-dentin bonding as an array. *J Dent Res* 1999;78:699-705.
 38. Yoshiyama M, Sano H, Ebisu S, Tagami J, Ciucchi B, Carvalho RM, *et al.* Regional strengths of bonding agents to cervical sclerotic root dentin. *J Dent Res* 1996;75:1404-13.
 39. Sirin Karaarslan E, Yildiz E, Cebe MA, Yegin Z, Ozturk B. Evaluation of micro-tensile bond strength of caries-affected human dentine after three different caries removal techniques. *J Dent* 2012;40:793-801.
 40. Ceballos L, Osorio R, Toledano M, Marshall GW. Microleakage of composite restorations after acid or Er-YAG laser cavity treatments. *Dent Mater* 2001;17:340-6.
 41. Banerjee A, Kidd EA, Watson TF. Scanning electron microscopic observations of human dentine after mechanical caries excavation. *J Dent* 2000;28:179-86.
 42. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, *et al.* Buonocore memorial lecture. Adhesion to enamel and dentin: Current status and future challenges. *Oper Dent* 2003;28:215-35.

43. Jepsen S, Açil Y, Peschel T, Kargas K, Eberhard J. Biochemical and morphological analysis of dentin following selective caries removal with a fluorescence-controlled Er:YAG laser. *Lasers Surg Med* 2008;40:350-7.
44. Sattabanasuk V, Burrow MF, Shimada Y, Tagami J. Resin adhesion to caries-affected dentine after different removal methods. *Aust Dent J* 2006;51:162-9.
45. Hossain M, Kimura Y, Nakamura Y, Yamada Y, Kinoshita JI, Matsumoto K. A study on acquired acid resistance of enamel and dentin irradiated by Er, Cr:YSGG laser. *J Clin Laser Med Surg* 2001;19:159-63.
46. Bertrand MF, Hessleyer D, Muller-Bolla M, Nammour S, Rocca JP. Scanning electron microscopic evaluation of resin-dentin interface after Er:YAG laser preparation. *Lasers Surg Med* 2004;35:51-7.

