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Can the Method of Primer Application Influence Adhesion to Er:YAG-laser Irradiated Dentin?

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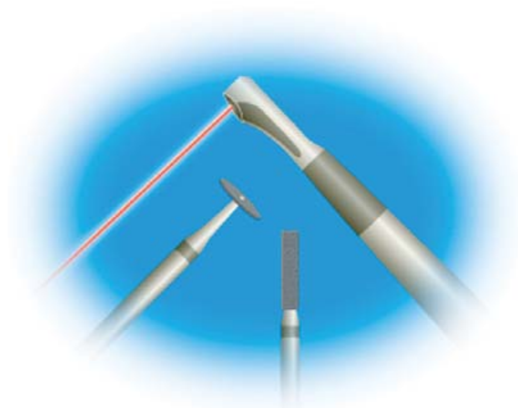

Abstract

Aim: The aim of this study was to evaluate how cavity preparation and primer application methods influence the adhesion of composite resin to dentin using a self-etching system.

Methods and Materials: Forty-eight extracted, sound human molars were divided into six groups (n=8) according to the method used for the surface preparation of the teeth (#600-grit paper disc, diamond bur, and Er:YAG laser) and the primer application method utilized (active or passive). Following the adhesive procedure using a self-etching system, 5 mm high composite buildups were created with Z-250 composite resin. After storage in water at 37°C for 24 hours, the specimens were vertically sectioned into serial 1 mm^2 sticks and tested for microtensile strength (μ-TBS). Nine additional molars were prepared for morphological analysis using scanning electron microscopy (SEM).

Results: Statistical analysis showed surface preparation technique (p<0.00) and primer application methods (p<0.001) do influence bond strength of the self-etching system tested. The best adhesion was achieved with specimens having the dentin ground with sandpaper followed by those ground with diamond burs. The worst bonding was obtained with Er:YAG laser ablated dentin. The active primer application method increased bonding performance compared to the passive method.

Conclusions: Within the limitations of the study, it can be stated surface treatment can influence the bond strength of the self-etching system tested and the active primer application method is more effective in achieving success in bonding to dentin compared with the passive application method.

Clinical Significance: Clinicians should use the active application method to apply a mild acidic self-etching primer along with an appropriate tooth surface preparation to facilitate the bond strength between dentin and composite resin.

Keywords: Adhesion, Er:YAG laser, dentin, scanning electron microscopy, SEM, primer application

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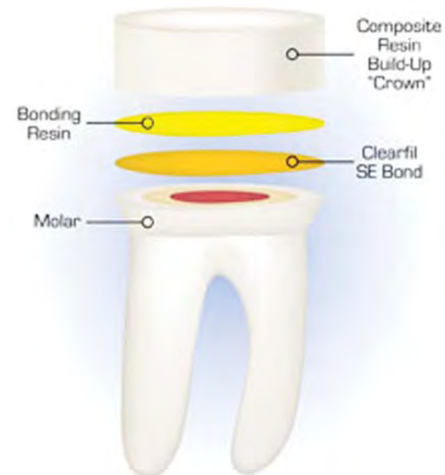
Introduction

The amount and quality of smear layer generated during cavity preparation varies according to the type of instruments used and due to its inherent frailty it is supposed to interfere with adhesion.[1] The thickness of smear layers formed during cavity preparation can interfere with the diffusion of acidic primers impairing the quality of the adhesion of self-etching systems.[3,4,5] As a result, the evaluation of different tools for cavity preparation and the smear layers they generate are of prime importance in adhesion studies.

Adhesive procedures have become less technique-sensitive and more simplified since the advent of self-etching systems. Such adhesive systems are composed of an acidic primer and a resin bonding agent that are available in either single-step or two-step procedure types. The acidic primer is able to penetrate the smear layer and bond to the underlying dentin. In a two-step procedure the bonding agent follows the primer which generates thin hybrid layers and short resin tags but with an acceptable adhesive performance.[2]

Miyazaki et al.[6] and Manhães et al.[7] have suggested an active application of the acidic primer can promote superior bonding when compared to the passive application of this component as proposed by their manufacturers. Using scanning electron microscopy (SEM) images they also showed active application of the acidic primer allows a greater primer infiltration into the underlying dentin tissue.[6]

Self-etching adhesive systems were developed to be used on tooth surfaces prepared by high-



speed cutting instruments. More recently, cavity preparations are performed using other methods such as laser irradiation. The application of wavelengths on dental tissues such as those associated with Er:YAG, Er,Cr:YSGG, Nd:YAG, CO_2, and Argon lasers have been studied. However, the erbium lasers (Er:YAG and Er,Cr:YSGG) hold the most promise for use in cavity preparations as it is highly absorbed by water within hard dental tissues. They are shown to be safe to dental pulp and to tissues adjacent to the irradiated area, producing temperature rises within acceptable limits (<5.5°C).[8-10] The Er:YAG laser has an antibacterial effect[10,11] considered to be a good feature when a smear layer is present on the dentinal surface. The patient also feels more comfortable during cavity preparation due to reduced noise and vibration which can lead to a decreased need for anesthesia.[9] The complete absence of a smear layer on irradiated dentin, its irregular surface, and open dentinal tubules suggests such a surface is conducive to favorable adhesion.[8-13] However, this matter is controversial due to some studies[14-18] demonstrating less adhesion to Er:YAG laser irradiated dentin, while others[19,20] reported improved adhesion under the same experimental conditions.

Microtensile bond strength testing is performed using a reduced interface area (approximately 1 mm^2) with beam-shaped specimens. Initial studies considered each individual beam as one experimental unit of the group. In this sense, with five or six teeth one was able to compose the sample for a study. This was the case when Manhães et al.[7] presented their results in 2005. Beams were considered the standard experimental units regardless of the number of teeth involved. Currently the experimental unit is considered the tooth. No matter how many beams can be obtained from each tooth, the arithmetic mean of the obtained beams derived from the tooth is representative of its final mean. The final mean of the experimental group is represented by the arithmetic mean of teeth that composed the group. This is considered a better experimental design that takes into consideration the substrate in order to obtain precise results.[21]

The aim of this *in vitro* study was to evaluate how cavity preparation technique and primer application methods can influence the dentin microtensile bond strength generated using a self-etching system and a contemporary approach to microtensile bond strength assay.

Methods and Materials

Microtensile Bond Test

This experiment was approved by the Research Ethics Committee (74/05). Fifty-seven extracted, sound human molars were used to compose the sample: forty-eight were used for μ-TBS testing and nine teeth were used for SEM evaluation. The sample was stored in distilled water at +4°C for less than six months after extraction.

The μ-TBS specimens were divided into six groups (n=8), according to type of surface treatment and primer application methods used to prepare them (Table 1).

Using a Ecomet 6/Automet polishing machine (Buehler Ltd., Lake Bluff, IL, USA) the occlusal surfaces of all samples were flattened perpendicular to their long axes with #120-grit sandpaper discs under a water coolant in order to expose dentin.[22,23] Then specimens assigned to Groups 1 and 2 were further prepared using #240 and #400 grit sandpaper discs to expose the superficial dentin followed with a disc of #600 grit sandpaper for 1 minute to obtain a standard smear layer recommended for laboratory testing.[24]

The dentin surfaces in Groups 3 and 4 were further prepared using a cylindrical diamond bur at high speed under a water coolant (18mL/min). The bur was positioned perpendicularly to the long axis in order to obtain a uniform surface. Each tooth was prepared using a new diamond bur.

Surface treatment of Groups 5 and 6 were carried out using a Er:YAG laser (KaVo KEY Laser 2, KaVo Co., Biberach, Germany). Laser usage and settings were as follows:

- Accomplished with 2051 handpiece
- Non-contact mode (12–15 mm distant from target tissue)
- Wavelength = 2.94 µm
- Spot size area of 0.63 mm^2
- Pulse width of 250–500µs
- Energy intensity of 250mJ/pulse for dentin[25]
- Frequency of 4Hz[25,26]
- Energy density of 80.3J/cm^2

Table 1. Experimental groups in study.

Primer Application Methods	Cavity Preparation		
	#600-grit paper disc	Diamond bur	Er:YAG Laser
Active	G1	G3	G5
Passive	G2	G4	G6

Table 2. Batch numbers, compositions, and application techniques of bonding agents studied.

Bonding Dystem	Batch Number	Composition	pH	Application Technique
Clearfil SE Bond	00330	Primer: MDP, HEMA, water, hydrophilic dimethacrylate	1.9	Apply primer and allow to act for 20 seconds; Briefly air-dry;
	00422	Bond: MDP, BIS-GMA, HEMA, water, hydrophilic dimethacrylate, microfiller	-	Apply bond, spread with gentle air stream and light-activate for 10 seconds.

BIS-GMA = bisphenyl-glycidyl-methacrylate; HEMA = 2-hydroxyethyl methacrylate; MDP = 10 – methacryloyloxydecyl dihydrogen phosphate.

Irradiation was conducted using a scanning motion perpendicular to the dentinal surface along with an air-water coolant at a flow rate of 24µl/min[13] and in compliance with all international safety rules (ANSI).

Immediately after surface treatments of the respective groups, the Clearfil SE Bond (SE) self-etching adhesive system (Kuraray, Osaka, Japan) was applied. In Groups 2, 4, and 6 the primer was passively applied according to the recommendations of the manufacturer. In Groups 1, 3, and 5 the primer was applied using the active method which consists of continuously scrubbing the primer over the dentin surface for 20 seconds.[6] The composition of the adhesive system is indicated in Table 2.

All groups were then lightly dried with an oil-free air syringe, and the bonding resin of the adhesive system was applied to create a thin, homogeneous pellicle that was photocured for 10 seconds with a halogen light curing unit (Astralis 3 - Ivoclar Vivadent, Amherst, NY, USA) at 600 mW/cm^2. Composite resin (Z-250) build-up "crowns" (3M Dental Products, St. Paul, MN, USA) were fabricated on the bonded surfaces in increments of 1 mm thickness and light activated for 30 seconds using the same parameters as was used for the adhesive system. Specimens were then stored in distilled water at 37°C for 24 hours prior to the sectioning procedure.[27,29]

After storage, the specimens were longitudinally sectioned serially in two-directions perpendicular to the adhesive interface using a Isomet 1000 diamond saw (Buehler Ltd., Lake Bluff, IL, USA), yielding approximately $1mm^2 \pm 0.10mm^2$ beams (Figure 1).[3,22]

The exact dimension of each sectioned beam was then individually measured using a digital caliper. The specimens were individually attached to a Geraldelli Jig[30] (Figure 2) with cyanocrylate resin for microtensile testing and subjected to a tensile force in a Model 4442 Inströn universal testing machine (Inströn Inc., Canton, MA, USA) at a crosshead speed of 0.5 mm/min.[27,31]

Bond strength was calculated in MPa. The means and standard deviation for bond strengths were obtained from each experimental group. Analysis of variance (ANOVA) and comparison of means by a Tukey interval at the 95% level of confidence were performed using Minitab 14 software (Minitab Inc. 14, State College, PA, USA).

Specimen Preparation for Fracture Analysis

After the μ-TBS test, debonded beams were glued side-by-side and stained using Ponceau S (2%), a protein-staining solution,[7,32] for 5 minutes and washed in running water for 10 seconds. The specimens were then examined at 20X magnification with a Citoval 2 microscope (Carl Zeiss-Jena, Standort Göttingen-Vertrieb, Germany) attached to a video camera (Digital Hyper HAD, Sony, Tokyo). Images were acquired using Captvator and VideCap 32 and processed by ImageLab 2000 (Canborough, Ontario, Canada). This imaging program is able to calculate image areas by pixels, thereby, obtaining percentage areas for each fracture type classified as follows:

- Cohesive failure of substrate (CS)
- Cohesive failure of restorative material (CR)
- Adhesive failure (AF)
- Mixed failure (specimens that presented more than one of the failure types mentioned) (MF)

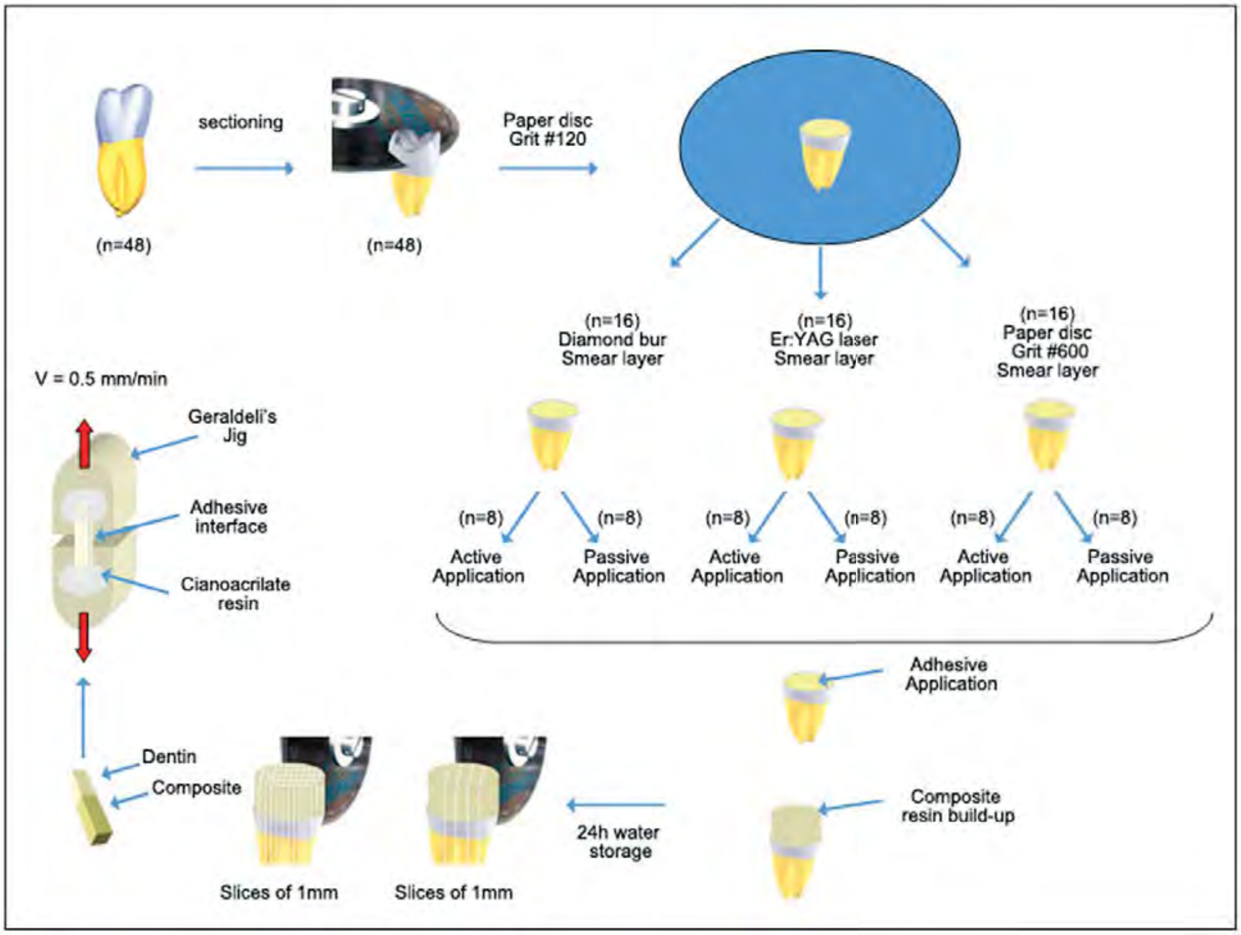


Figure 1. Specimen preparation.

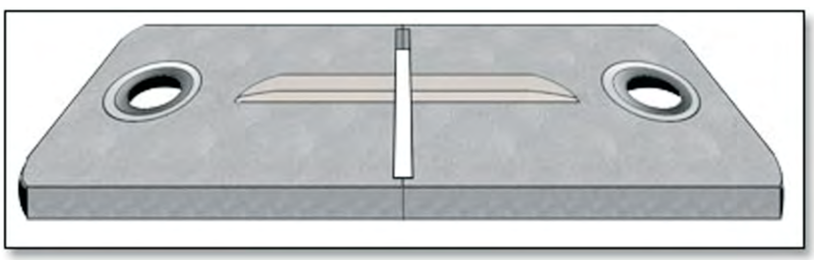


Figure 2. Geraldelli's Jig used for the microtensile test.

SEM Preparation

Nine occlusal superficial dentin discs obtained from sound human molars were used to create the samples. Each dentin disc was divided into two equal sections in order to repeat the procedure and verify the accuracy of obtained results. The resultant specimens were prepared as follows:

1. #600 - grit sandpaper disc for 1 minute (SP)
2. SP + active primer
3. SP + passive primer
4. High-speed diamond bur (DB)
5. DB + active primer
6. DB + passive primer
7. Er:YAG laser irradiation (ER)
8. ER + active primer
9. ER + passive primer

Immediately after preparation, the discs were immersed in 2.5% glutaraldehyde for 12 hours at +4°C. Samples were dehydrated in ascending

Table 3. Descriptive statistics of bond strength obtained on experimental groups.

Group	n	Mean * (MPa)	SD(±)	Mean error
1	8	47.57 a	2.80	0.99
2	8	47.16 a	4.84	1.71
3	8	31.20 b	2.68	0.95
4	8	26.54 c	3.09	1.09
5	8	26.21 d	3.18	1.12
6	8	21.23 e	0.95	0.34

*Means with the same letter are statistically the same.

Table 4. Specification for fracture on tested groups.

Groups	CS (%)	CR (%)	AF (%)	MF (%)
G1	3.08%	1.54%	92.30%	3.08%
G2	6.77%	0%	93.23%	0%
G3	0%	0%	98.44%	1.56%
G4	2.47%	0%	87.65%	9.88%
G5	0%	1.56%	93.75%	4.69%
G6	0%	0%	100%	0%

concentrations of ethanol (50–70–95–100%). Thereafter, specimens were gold sputter-coated and the tested dentin surfaces were examined using SEM (JEOL-JXA-6400, Tokyo, Japan) at a magnification of 2000X.

Results

Microtensile Bond Test (μ-TBS)

Descriptive statistics of bond strength of tested groups are described in Table 3. The general linear model of ANOVA detected statistical differences for the main factors surface preparation ($p<0.05$) and primer application ($p<0.01$), while the interaction of the main factor suggest a non-significant statistical result ($p=0.083$).

The best surface preparation for achieving the highest adhesive resistance was obtained by using #600-grit sandpaper discs. The next highest was achieved using diamond burs, while the lowest adhesion values were observed with the Er:YAG laser ablated dentin. In order to reach high dentin bonding performance the active primer application was more effective than the passive application method.

Fracture Evaluation

Table 4 presents the results for fracture evaluation. All tested groups exhibited mainly adhesive fractures, although some had different percentages that did not compromise the final results.

SEM Observations

When #600-grit paper discs were used, a typical, homogenous image of smear layer could be observed (Figure 3).

When the primer was actively scrubbed over the dentinal surface, the smear layer was visibly altered. The smear plugs were partially dissolved and an amorphous structure could be seen. This structure might be composed of either hydroxyapatite crystals or a chemical residue of the component of the primer (Figure 4).

However, when the primer was passively applied, the smear layer and smear plugs were preserved on the dentinal surface (Figure 5).

When dentin was prepared with a diamond bur at high speed, a smear layer with a heterogeneous and granular aspect was observed (Figure 6).

After active primer application over the dentin surface, dentinal tubules apertures could be seen suggesting the smear layer had been partially dissolved (Figure 7). However, some structures remained on the surface which could correspond to precipitated hydroxyapatite crystals or a chemical component of the primer.

A greater amount of smear layer covered the dentinal surface passively treated with the primer solution. The smear plugs remained in position suggesting a partial removal of the smear layer (Figure 8).

Open dentinal tubules, an irregular surface, free of a smear layer and smear plugs are typical features of Er:YAG laser irradiated dentin (Figure 9). Peritubular dentin is preserved along with the presence of a significant mineralized component.

When the primer was actively applied, the presence of a great amount of precipitated hydroxyapatite or chemical components of the primer covering the surface was observed which impaired the visualization of peri- and inter-tubular dentin and even the dentinal tubules (Figure 10).

With a passive application of the primer the dentinal tubules, peri- and inter-tubular dentin could be clearly observed with a small degree of amorphous structure remaining on the surface (Figure 11).

Discussion

Cavity preparation instruments leave a layer of dental debris referred to as the smear layer which varies in composition with the type of instrument used. The smear layer is described in the dental literature[3,5] as having distinct characteristics

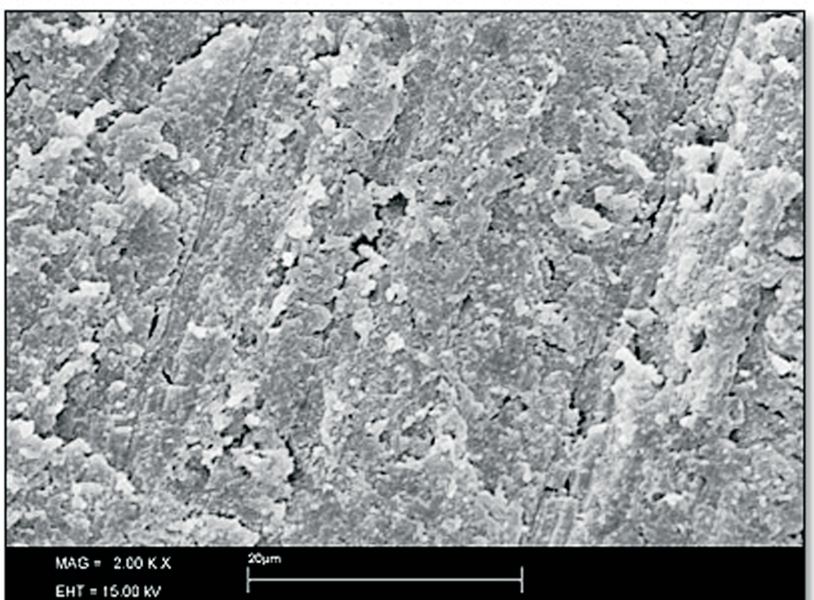


Figure 3. #600-grit paper disc control specimens showing a compact smear layer, while dentinal tubules apertures cannot be seen.

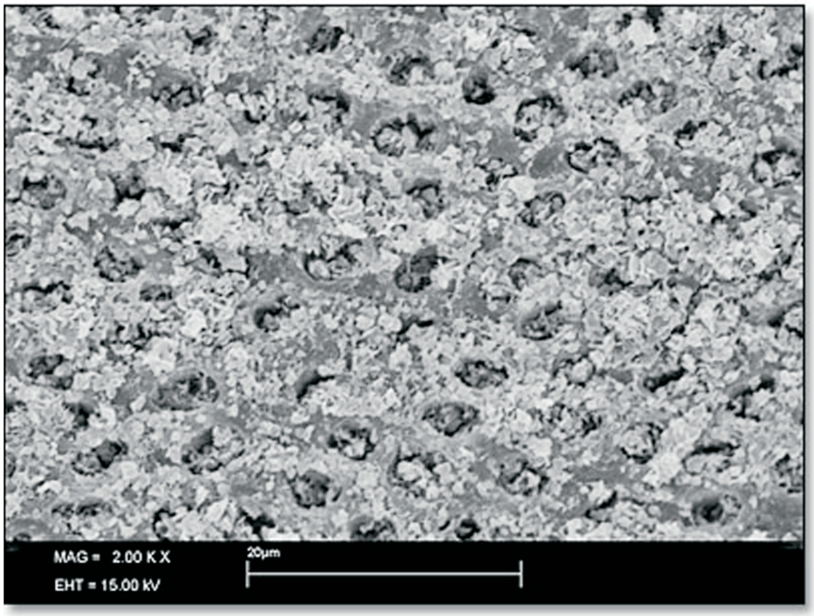


Figure 4. Primer actively applied over dentin treated with #600-grit paper discs originate little amount of smear layer. Smear plugs are present obstructing dentinal tubules, as well as intertubular dentin, and amorphous structures, suggesting the presence of remaining hydroxyapatite crystals or chemical components of the primer.

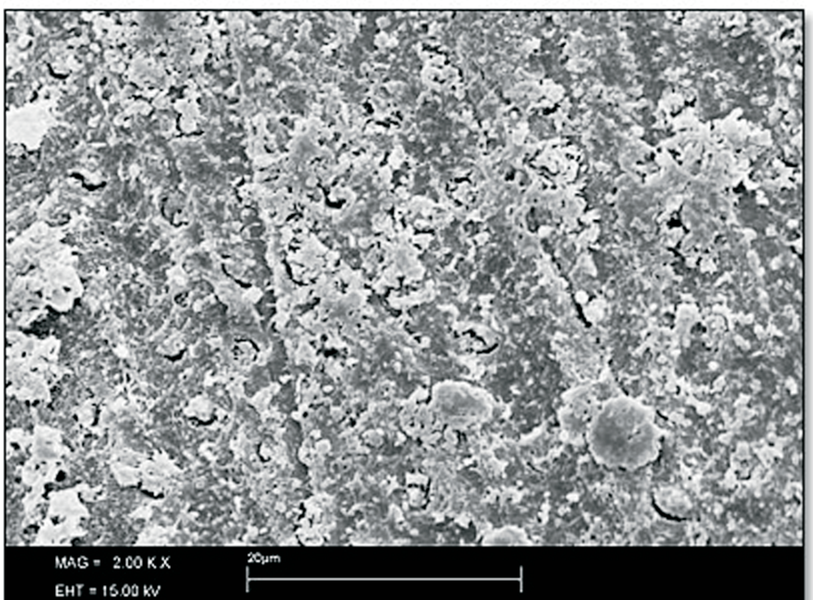


Figure 5. Passive application of the primer over dentin abraded by #600-grit sandpaper discs maintained the smear layer on the surface and smear plugs are observed in their original positions.

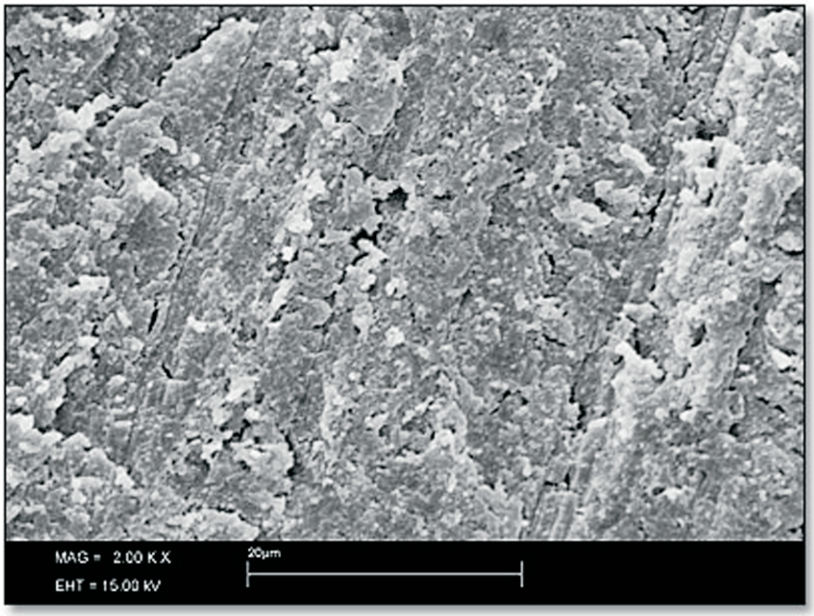


Figure 6. Dentin surface grounded by diamond bur covered by a heterogeneous smear layer with granular aspect.

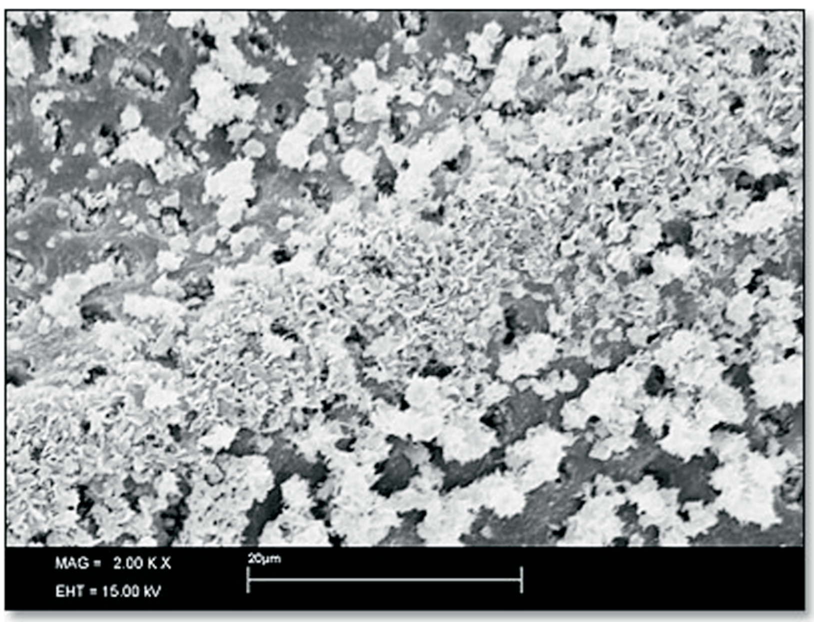


Figure 7. Specimens prepared with a diamond bur at high speed with active primer application of the primer showing amorphous structures suggesting hydroxyapatite crystals or chemical components of the primer solution.

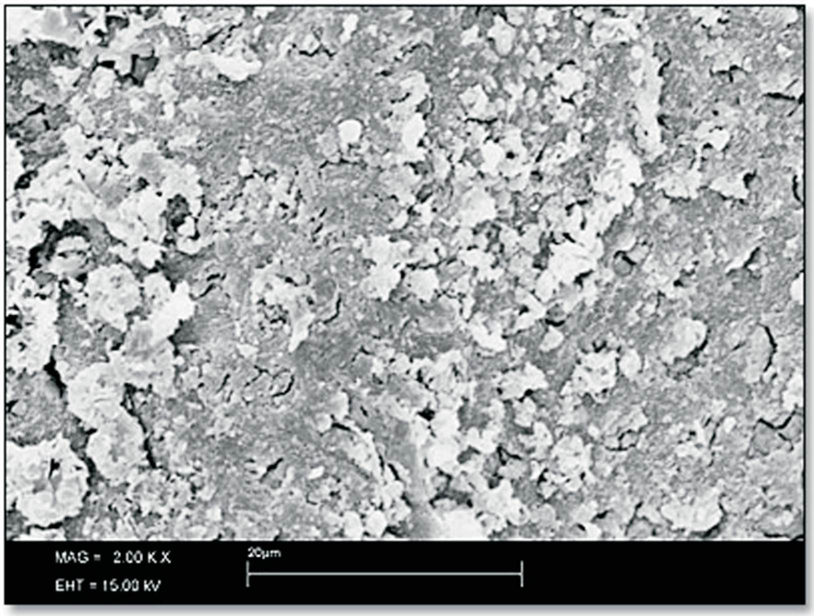


Figure 8. Passive primer application over diamond bur treated dentin maintains significant amount of smear layer with granular appearance at its original position as well as smear plugs.

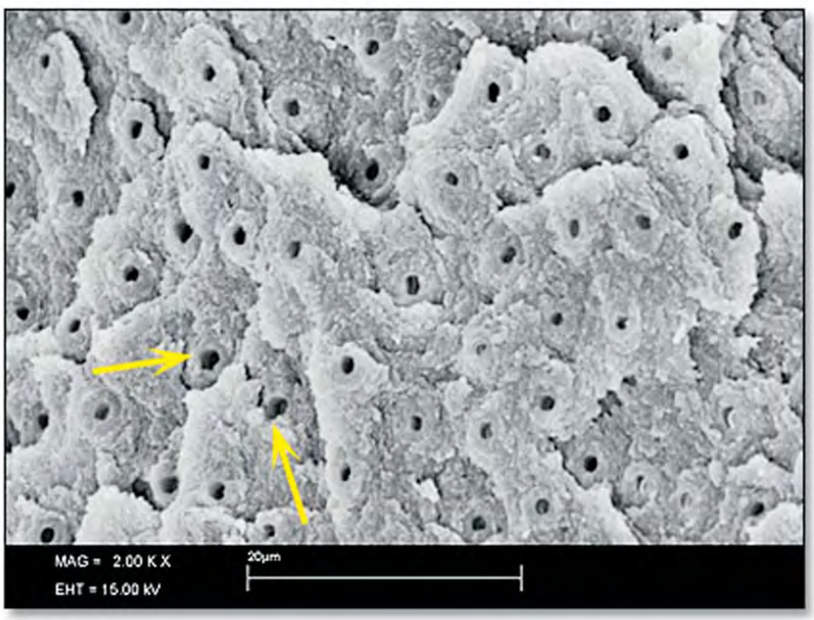


Figure 9. Typical Er:YAG ablated irregular dentin, with open dentinal tubules (arrows), no smear layer or smear plugs.

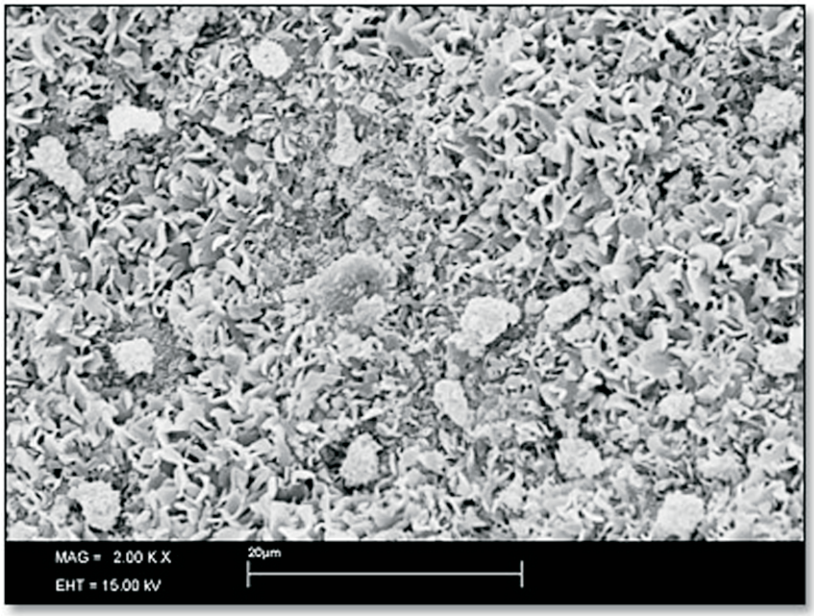


Figure 10. SEM microphotographs of Er:YAG laser specimens showing a spinelike structure obstructing dentinal structures suggesting the presence of remaining hydroxyapatite crystals or chemical components of the primer.

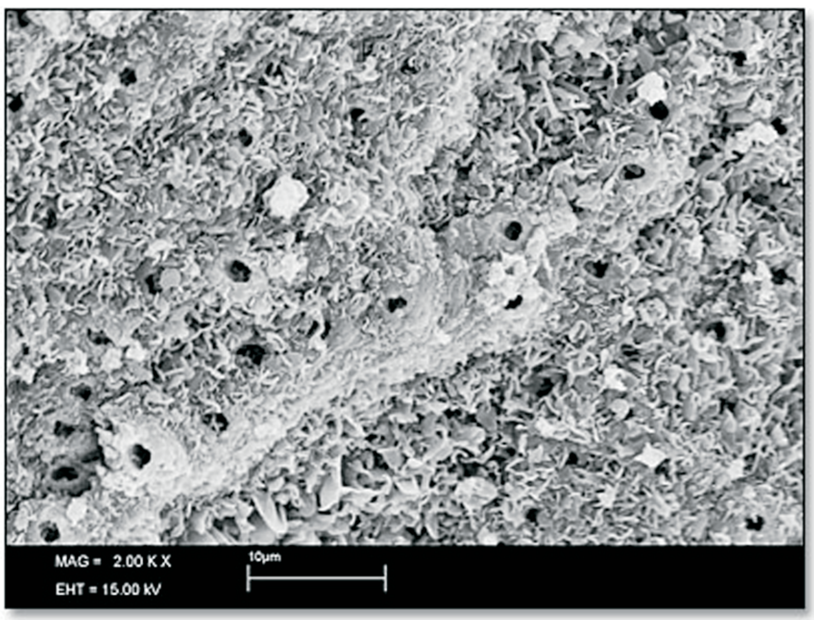


Figure 11. When Er:YAG laser irradiated dentin had the primer passively applied, a large number of dentinal tubules can be seen with amorphous structures over the surface. At the same time, a large number of dentinal tubules remain open.

related to the type of instrument used, the use of water spray, and the pressure applied to the instrument during cavity preparation. Diamond burs create a thicker layer compared to carbide burs while a thinner smear is obtained using hand instruments.[33,34] The Er:YAG laser has been suggested as an alternate means of removing caries and creating the cavity preparation. Such a strategy raises questions about the nature of the morphological characteristics of the tooth surface irradiated by Er:YAG laser and whether they are distinct from the characteristics obtained using conventional preparation methods. This knowledge is essential to achieve a successful outcome for adhesive restorations that are routinely fabricated in daily practice.

Dentin ablated by an Er:YAG laser presents open dentinal tubules,[8,10,13] a complete absence of a smear layer, and morphological features distinct from high and low speed conventional cavity preparation.

The importance of the thickness of the hybrid layer in order to obtain adhesive success especially when self-etching adhesive systems are used has been reported.[35] Others have reported the denseness of smear layer should also be taken into consideration.[36]

Due to the importance of these issues in adhesive dentistry this study tested the adhesive performance of a mild pH two-step self-etching primer applied to dentinal surfaces that were prepared three ways: using #600-grit sandpaper discs, using a high speed diamond bur, or using an Er:YAG laser. This study also investigated the effect of the method of application of a mild acidic primer in an effort to determine if an active method would be more effective in the permeation of the primer through the smear layer compared to a passive application.

The results showed the different surface preparation methods influenced bond strength and the active method of primer application increased bond strength.

The dentinal surfaces that were best permeated by the adhesive system was created by using #600-grit sandpaper discs. This is considered as the gold standard surface for bonding,[37] and the results of the present study is in accordance with previous studies.[5,7,29]

The SEM micrographs of the specimens prepared using sandpaper discs in the present study showed a homogenous smear layer (Figure 2) when compared to that obtained with diamond burs (Figure 5). In this context, it can be assumed the self-etching primer could easily permeate this layer and bond to the subjacent dentin. However, sandpaper discs are not instruments that are suitable for clinical cavity preparations,[36] but they do produce a gold standard surface for experimental studies.[37] Therefore, a primary goal is to develop clinical instruments for cavity preparation that creates a similar smear layer on the dentinal surface in an effort to obtain favorable adhesion.

Using high-speed diamond burs produces lower adhesion when compared to sandpaper discs. They generate a large quantity of granules and a smear layer with a heterogeneous aspect that impairs the visualization of the dentinal tubules apertures and the distinction between peri- and inter-tubular dentin (Figure 5). The present study is in agreement with other studies claiming this remaining heterogeneous surface to be difficult for a self-etching primer to permeate.[33,34]

Like Kameyama et al.[38] the present study found Er:YAG laser irradiated dentin to have the lowest adhesion values when compared to sandpaper discs and diamond burs.[38] However, the findings of Manhães et al.[7] completely disagree with these results for they found similar bond strengths using diamond burs and the Er:YAG laser to prepare dentin. This could be accounted for based on sample selection alone.

The SEM micrographs of Er:YAG laser irradiated dentin in the present study presented complete absence of smear layer and smear plugs, irregular surface with deep and shallow areas, and clearly visible dentinal tubules apertures as well as peritubular dentin (Figure 8) which is in accordance with the description of ablated dentin in the dental literature.[8,12,38-40]

Conventional adhesion concepts suggest exposed tubule apertures can increase adhesion, but this was not observed in this study. Therefore, it can be assumed other alterations, rather than morphological, are created when dentin is irradiated by an Er:YAG laser resulting in a change of the dentin structure that resists mild acid demineralization.

Er:YAG laser can be used in dentistry for different purposes, but an in depth understanding of the morphological and chemical features of ablated dentin is lacking. With regard to bonding, more studies are needed to investigate the development of new adhesive systems able to adequately interact with irradiated substrates. Such studies will be of prime importance in terms of the use of Er:YAG laser in operative dentistry.

The findings of the present study related to surface preparation is in agreement with others[29,36] noting the importance of using appropriate instrumentation for cavity preparation. A finishing procedure using multilaminate burs should be of routine use, especially when self-etching adhesive systems will be applied. [29]

In the present study the active application was found to promote better adhesion than the passive method which is in accordance with Miyazaki et al.[6] but in complete disagreement with Manhães et al.[7] based on their sample selection.

Previous studies[1,27] using microtensile bond strength tests considered beams as experimental units obtained from a small number of teeth. However, Reis et al.[21] demonstrated specimens should not be considered as experimental units, due to the possibility of affecting original data independence, impairing usual statistical analysis (ANOVA and comparison of means by a Tukey interval) which presuppose independent data. This statistical concern was taken into account in the present study with regard to sample selection, and eight teeth were used for each group.

The composite-substrate interface is a complex structure with numerous potential fracture sites.[41] The final pattern of fracture types of the adhesive interface is determined by the degree of stress at the moment of the test, by crack propagation; by properties of the material being tested; and by the dynamic of the fracture event itself.[41] Thus, the most fragile portion of the interface can or cannot be included in the fracture modes studied. Moreover, Hashimoto et al.[41] believe the way that fractures occur is related to differences in the methodology of the adhesion tests or to the shape of the specimens.

It is considered of fundamental importance to study the relationship between the adhesive test methods used, resistance data obtained, and the types of fracture because there is a lack of correlation between bond strength data and fracture types in the literature.[41]

In this study, when fracture types were analyzed (Table 4), the predominance of adhesive fractures validated the method used to test bond strength. The low number of cohesive substrate fractures demonstrated the experimental design used to compose the groups and the efforts related to sample collection were fundamental for the success of this study. Moreover, using a trained professional in microtensile strength testing is fundamental to determine the low occurrence of cohesive failures of a restorative material for they can also occur when the cohesive strength of the material is lower than the adhesive strength.

For now, the absence of a correlation between adhesion data and fracture type remains a reality. Some investigators[42] have attempted to establish some kind of relationship between these data performing a SEM analysis of fractured sticks or by taking into consideration the fracture type when mean values of bond strength are calculated using a specific index for microtensile bond strength.[43] Despite these efforts a correlation has not yet been established as a research protocol.

The usual way to present microtensile bond strength data is to show obtained means that have been submitted for statistical analysis and fracture data are presented in percentage values. Other studies need to be conducted in order to develop a new method of analysis that takes into consideration numerical and morphological data.

Conclusions

Based on obtained microtensile bond strength results, the following conclusions can be stated:

1. Surface preparation strategies such as the use of sandpaper discs, diamond burs, and Er:YAG laser create dentinal surfaces with completely distinct morphological features that influence the adhesive resistance of the tested self-etching system.
2. Considering the clinical testing methods, better adhesion was obtained in surfaces that were prepared by diamond burs, than dentin irradiated with the Er:YAG laser.
3. The active method of primer application was more effective than the passive method.

Clinical Significance

Clinicians should use the active application method to apply a mild acidic self-etching primer along with an appropriate tooth surface preparation to facilitate the bond strength between dentin and composite resin.

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