Effect of caries removal techniques on the bond strength of adhesives to caries-affected primary dentin in vitro

ABSTRACT

Aim The aim of this in vitro study is to evaluate the effects of three different caries removal techniques on the microtensile bond strength of adhesive materials to caries-affected dentin.

Materials and methods Thirty primary molar teeth were used. The teeth were randomly divided into three groups according to the caries removal technique employed: conventional steel bur (group 1), Er:YAG laser (group 2); chemomechanical method (group 3). Each group was divided into two subgroups according to bonding agents: one-step self-etch adhesive and etch-and-rinse adhesive. The teeth were restored with composite resin. Vertical sticks were obtained and subjected to tensile stress. Data were analyzed by two-way analysis of variance (ANOVA), Tukey’s test and an independent samples t-test.

Results The values for the laser groups were significantly lower than those of the bur groups for both bonding agents (p<0.05). There were no significant differences between the bur and chemomechanical groups (p > 0.05).

Conclusion Bur and chemomechanical techniques in primary teeth were found more successful. Similar results were found according to the adhesives used for each caries removal techniques.

Introduction

Dental caries must be treated with removal of carious tissues and restoration of missing tissue. Removal of caries is conventionally performed by rotary or non-rotary mechanical techniques. Indeed, the ideal cutting instrument and technique should have properties that satisfy both dentist and patient, such as the following: easy to maintain and use, economical, require minimal pressure, discriminate and remove only caries-infected tissue, produce no vibration or heat during the removal process, painless and silent for patient comfort.

The use of burs is popular worldwide but usually causes discomfort and pain, which is associated with sensitivity of vital dentine, pressure on the tooth, noise of the air turbine handpiece and development of heat and vibration. The advantage of the rotating bur is that it reduces the overall treatment time because it cuts carious dentine easily.

In paediatric dentistry, the cooperation of children is an important issue. Pain, drilling, and local anaesthesia were reported to be the most frightening aspects. Local anaesthetic may be required for the use of the rotary handpiece and bur in almost all cases of deep caries and the pressure of drilling is unpleasant. However, alternative techniques have been introduced, and in recent years, lasers and chemomechanical techniques have also been used for the removal of carious tissue.

Carisolv, which is the chemomechanical caries removal system, includes sodium hypochlorite (0.95% NaOCl) and 3 amino acids (lysine, leucine, and glutamic acid) in a gel preparation. The softened carious dentin can thus be removed. This technique was found advantageous because the need for local anaesthesia is reduced. Furthermore, no reduction in operating time was observed.

When the lasers were developed, hard tissue lasers came into use in 1997. The erbium laser’s shallow depth of tissue penetration, lack of thermal damage, and minimal reflective property make it a safe and efficient laser for paediatric dentistry. Hard tissue lasers have an impressive ability to reduce or eliminate vibration, the audible whine of drills, microfractures, and some of the discomfort that many patients fear and commonly associate with high-speed handpieces. Because disinfecting the cavity is desirable to prevent residual caries and pulp inflammation, laser applications are advantageous because of the destruction of bacteria in the dentin.

The technique chosen for caries removal produces different dentin surface characteristics. The dentin
ultrastructure affects the bonding of adhesives materials. Previous studies focused on several caries removal techniques. The present study compares the use of bur, laser and a chemomechanical removal technique in primary teeth. The aim is to evaluate the microtensile bond strength (μTBS) of etch-and-rinse and one-step self-etch adhesives to caries affected dentin surfaces treated with various caries removal techniques.

The null hypotheses investigated in this study are as follows:
› There are differences among the bonding values of 3 different caries removal techniques (conventional bur, Er:YAG laser and chemomechanical removal/Carisolv).
› There are no differences in the bonding values of etch-and-rinse and one-step self-etch adhesives.

Methods
Selection of teeth and caries removal
The study protocol was approved by the Ethics Committee of the University of Gaziantep and the study was performed at the University of Gaziantep. Thirty primary molar teeth with caries lesion (therapeutically extracted in cases of serial extractions, pre-shedding mobility) were collected and teeth with caries lesion extending at least half of the distance from the enamel-dentin junction to the pulp chamber were included in the study. All teeth were stored at 4 °C in physiological saline for a maximum of four weeks. Enamel and superficial dentine of the crown were flattened perpendicular to the long axis of the tooth with bur until the lesions showed laser fluorescent values of approximately 40–50 (Diagnodent, Kavo Dental, Biberach, Germany). After the specimens were washed with deionized water for 1 minute, the teeth were randomly divided into three groups according to caries removal technique: bur (group 1), Er:YAG laser (group 2), chemomechanical removal (group 3), and each group was divided into two subgroups (n = 5) according to the adhesive system used (etch-and-rinse or one-step self-etch).

In the bur groups, caries was removed with a round steel bur (No. 14, 16 Gebr Lemgo, Germany) with a water cooled, slow speed hand piece (Bien Air, S/N 09B0600, Switzerland).

In the laser groups, an Er:YAG laser system (Fidelis Plus III, Fotona) with a laser wavelength of 2.94 μm was used to remove the caries. Power output of 3.5 W, pulse duration of 300 μs (short pulse [SP] mode); the pulse repetition rate was 10 Hz. Irradiation of a focused beam was performed at distance of 1 mm (energy density: 44 J/cm²). Cylindrical quartz with a diameter of 1 mm (65320, Fidelis Plus III, Fotona) was mounted to the R14 handpiece for dentin ablation. The irradiated area was cooled continuously by using an air and water spray system.

In the chemomechanical removal group, caries was covered with Carisolv gel Multimix (MediTeam, Göteborg, Sweden). After 30 seconds, the carious lesion was gently excavated with the Carisolv metal curettes (20102 Carisolv Instrument Kit; MediTeam Dental AB, Sävedalen, Sweden) to remove softened carious tissues. The remaining gel was cleaned with wet and dry cotton pellets.

For each technique, visual observation and tactile examination by probing, that rely on subjective clinical criteria (such as color and softness), were performed and removal of the carious lesion was repeated until the laser fluorescence value decreased to approximately 20 in the centre of the lesion, so that the infected dentin was completely removed.

An etch-and-rinse (Adper single bond 2, 3M Dental Products, MN, USA) and a one-step self-etch (G-bond, GC Corporation, Tokyo, Japan) adhesive were used for the study. Adhesives were applied in the line of caries-affected dentin surface according to the manufacturer’s instructions (Table 1). Following the application of adhesives, composite resins of bonding agents (Filtek Z 250, 3M ESPE dental products, St. Paul, USA; Gradia

<table>
<thead>
<tr>
<th>BONDING SYSTEMS</th>
<th>MANUFACTURER</th>
<th>COMPOSITION</th>
<th>APPLICATION MODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adper single bond 2 (etch-and-rinse)</td>
<td>3M Dental Products, MN, USA</td>
<td>35% phosphoric acid bis-GMA, HEMA polyalkenoic copolymer ethanol, purified water</td>
<td>Etch substrate for 15 s, rinse with water spray and dry gently apply bonding resin, air-thin, light cure for 10 s</td>
</tr>
<tr>
<td>G-Bond (One-step self-etch)</td>
<td>GC Corporation, Tokyo, Japan</td>
<td>4-MET, UDMA, dimethacrylate component, phosphoric ester monomer, acetone, water</td>
<td>Apply bonding resin, left undisturbed for 10 s, dry for 5 s with maximum air pressure and light-activate for 10 s</td>
</tr>
</tbody>
</table>

Abbreviations: Bis-GMA, bisphenyl-glycidyl-methacrylate; HEMA, 2-hydroxyethyl methacrylate; 4 MET, 4-methacryloxy ethyltrimellitic acid UDMA, urethane dimethacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate.
Effects of caries removal techniques on adhesives bond strength

European Journal of Paediatric Dentistry vol. 14/3-2013

Table 2: Two-way analysis of variance (ANOVA).

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>DF</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bondings</td>
<td>3.451</td>
<td>1</td>
<td>3.451</td>
<td>0.140</td>
<td>0.710</td>
</tr>
<tr>
<td>Caries removal techniques</td>
<td>359.122</td>
<td>2</td>
<td>179.561</td>
<td>7.262</td>
<td>0.001</td>
</tr>
<tr>
<td>Bondings * Caries removal techniques</td>
<td>2.172</td>
<td>2</td>
<td>1.086</td>
<td>0.044</td>
<td>0.957</td>
</tr>
<tr>
<td>Error</td>
<td>2076.978</td>
<td>84</td>
<td>24.726</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34182.049</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>2441.723</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FIG. 1 Graphic of percentage distribution of the three different failure modes in the primary teeth.

Microtensile test
The teeth were vertically sectioned both mesiodistally and buccolingually along their long axis with a slow-speed diamond saw to obtain three 1 mm² stick-shaped microtensile specimens of which half were composite and half were dentin from each tooth (n = 15). Thicknesses of specimens were measured using digital calipers (Mitutoyo, Tokyo, Japan). These were fixed with cyanoacrylate glue (Zapit; DAVA, Corona, CA, USA) to two surfaces on a linear actuator-driven offset micro-tensile testing device (BISCO; Schaumburg, IL, USA) and were stressed at a crosshead speed of 1 mm/min until failure. The micro-tensile bond strength (μTBS) was expressed in MPa.

Failure mode
After the μTBS test, the fracture surfaces were examined with an optical microscope (Leica Microscopy Systems, Germany) under 40x. The failure modes were classified as follows: adhesive failure if 100% of the bonded interface failed between the dentin and the bonding resin; cohesive failure if 100% of the failure was in the resin composite or in the dentin; or mixed failure if the failures were partially adhesive and partially cohesive.

Evaluation using scanning electron microscopy (SEM)
Three additional molar teeth with dentinal caries were treated with the caries removal techniques, and the micromorphologies of the caries-affected dentine surface were evaluated by SEM (EVO LS10, Zeiss, Oberkochen, Germany). These substrates were fixed in 2.5% glutaraldehyde in phosphate-buffered solution 0.1 M for 24 hours at room temperature. Specimens were dehydrated in ascending grades of ethanol and submitted to chemical drying in hexamethyl disilazane. The specimens were gold sputter-coated, and caries-affected dentin surfaces were observed at SEM. The entire surface was scanned, and the most representative areas were photographed at 2,000x magnification.

Statistical analysis
The results of the Levene statistics (P > 0.05) and the Shapiro-Wilk statistics in all groups (p> 0.05) demonstrated variance homogeneity. The bond strength data were statistically compared by two-way analysis of variance (ANOVA), Tukey’s test, and an independent samples t-test. A Chi-square test was used to compare the incidence of the different fracture modes among the caries removal techniques. The data were analysed with SPSS 13 statistical program software for Windows (SPSS Inc., Chicago, IL). The level of significance was set at 5% (p< 0.05).

Results
Microtensile bond strength (μTBS)
The ANOVA results, showed significant differences in μTBS among caries removal techniques (p= 0.001) but there were no significant differences in the adhesive
systems (p= 0.710) (Table 2). The mean μTBS values and standard deviations for the experimental groups are shown in Table 3.

Irrespective of the bonding agents, the mean μTBS values of the bur and chemomechanical groups were higher than the laser groups. In each caries removal techniques, no significant differences were found between etch-and-rinse and self-etch adhesive systems (p> 0.05).

In the primary teeth groups, for both self-etch and etch-and-rinse adhesives, there were significant differences between the bur and laser groups (p< 0.05), while Carisolv was similar for both (p> 0.05) (Table 3).

**Failure modes**

Figure 1 shows the distribution of failure modes (adhesive, cohesive and mixed) for the different caries removal techniques and adhesive systems in the primary teeth. Most of the groups showed adhesive failure rather than cohesive failure and mixed failure. According to the Chi-square test, there were no statistically significant differences in failure modes among the groups (p> 0.05).

**Scanning electron microscopy**

SEM micrographs of the primary dentin after bur, laser and chemomechanical caries removal are shown in Figures 2 to 4.

After removal of the caries with burs, the dentine was covered by a smear layer, partially masking the dentinal tubules (Fig. 2). There was a residual smear layer on the dentine surface with dentinal tubules remaining occluded with smear plugs. This surface was irregular (original magnification 2000×).

The dentine ablated by the Er:YAG laser presented open dentinal tubules distributed on a scaly surface free of a smear layer, with intertubular dentine more ablated than the peritubular dentine (Fig. 3). The surface was generally free of smear layer and accompanied by open dentinal tubules and an irregular and microretentive morphological pattern. Collagen fibrils were also visible (original magnification 2000×).

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**TABLE 3** The μTBS values (MPa) (mean ±SD) of groups.

<table>
<thead>
<tr>
<th>Caries Removal Technique</th>
<th>Etch-and-Rinse System</th>
<th>One-Step Self-Etch System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotary bur</td>
<td>20.77±5.64bA</td>
<td>21.05±5.19bA</td>
</tr>
<tr>
<td>Laser</td>
<td>15.70±5.87aA</td>
<td>16.51±4.91aA</td>
</tr>
<tr>
<td>Chemomechanical</td>
<td>19.29±4.30abA</td>
<td>19.37±3.56abA</td>
</tr>
</tbody>
</table>

Same lowercase letters in same column indicate no statistically significant differences. Same capital letters in same row indicate no statistically significant differences.
In primary teeth, the caries-affected dentine treated with Carisolv showed a smear layer, which caused plugs within the tubular orifices. Some areas of open tubules were also observed (Fig. 4) (original magnification 2000×).

Discussion

Studies to evaluate an appropriate method for caries removal have been performed, because the techniques used have both disadvantages and advantages. Different caries removal techniques may affect the surface characteristics of dentin which can affect the bond strength of dental materials. Thus, we used irradiation with an Er:YAG laser and a chemomechanical method using Carisolv to evaluate bond strength values in primary teeth. The results obtained in the present study support the hypothesis that there are differences among the bond strength values of three different caries removal techniques (bur, Er:YAG laser and chemo-mechanical removal) and that there are no differences in the bonding values of etch-and-rinse and one-step self-etch adhesives.

A laser fluorescence (LF) device could be effective in evaluating residual caries. LF should be used with an additional diagnostic method. After the laser irradiation to dentine, the application of a caries-detecting liquid requires caution. Even after all the caries-affected dentine has been removed, a pink stain was reported in the dentine surface. This would probably lead to over treatment and over cutting of the dentine surface. The literature includes few studies that measured the reliability and validity of laser fluorescence in assessment of residual caries after caries removal. Using LF for evaluating residual caries is a methodological limitation of this in vitro study.

The adhesion mechanism of newer-generation dentin adhesive systems is thought to be related to the creation of a hybrid layer. It has been suggested that primary dentin is more susceptible to acid conditioning. Moreover, dentin hardness, dentin permeability, degree of mineralization, and adhesive systems may affect bond strength to dentin. The vaporisation of water and other hydrated organic components of the primary teeth dentin after the Er:YAG laser radiation results in an increase of the internal pressure, which leads to rupture of the dental structure. Thus, the bond values of laser groups may be lower than those of the bur groups. In the SEM images, only Er:YAG laser-prepared dentine (Fig. 3) showed open dentinal tubules without a smear layer with protruded peritubular dentine with a scaly surface, as observed in other studies. While these characteristics are considered ideal because of the increased adhesive area, Ariyaratnam et al. [1999] reported that there was no significant difference between the bonding value of resin composite to the laser-treated dentin and the untreated dentin, despite the fact that the surface roughness of the laser-treated dentin was significantly higher than the untreated dentin.

Monghini et al. [2004] reported that laser application decreased bond values, in agreement with the finding of the present study. In contrast to the present study, Fluery et al. [2011] reported that dentin of primary molars treated with Er:YAG laser had bond strengths similar to those obtained with diamond bur-treated dentin.

Conclusions

Within the limitations of this study, the following conclusions can be drawn.

- The technique used to remove the caries influenced the bond strength in caries-affected primary dentin in both etch-and-rinse adhesive and self-etch adhesive groups.
- It may be better to choose the caries removal method—either chemomechanical or rotary bur—according to the level of patient’s cooperation. However, further investigations with a greater sample size are needed to validate the outcomes of the present study.
References

- Lizarelli RZF, Moriyama LT, Jorge JRP, Bagnato VS. Comparative ablation rate from a Er:YAG laser on enamel and dentin of primary and permanent teeth. Laser Physics 2006;16:849-858.