The Efficacy of Different Sealer Removal Protocols on the Microtensile Bond Strength of Adhesives to a Bioceramic Sealer-Contaminated Dentin

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Research Article

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Abstract

**Background:** The optimal bonding of adhesives to dentin requires the sealer to be completely removed from dentinal walls. This study compared the efficacy of different sealer removal protocols on the microtensile bond strengths (MTBS) of single step adhesives to a calcium silicate-based bioceramic root canal sealer contaminated dentin.

**Methods:** Standardized box-shaped Class I cavities were prepared in human lower third molars (N=50). All cavities were contaminated with a bioceramic root canal sealer (Endosequence BC Sealer, Brasseler, Savannah, USA), except the control group cavities. For the experimental groups, dentin surfaces were wiped with dry cotton pellets, cotton pellets saturated with water or rinsed with an air water syringe for 5 seconds, or applied aqueous ultrasonic scaler for 5 seconds prior to restoration procedure. All the cavity surface was restored with a one-bottle universal adhesive and composite resin. All the specimens were subjected to both thermocycling and mechanical loading. The restored specimens were sectioned into resin-dentin beams for microtensile bond strength (Mtbs) evaluation. Additional specimens were prepared for scanning electron microscopy (SEM) to examination of dentin-adhesive interface (N=10).

**Results:** The Mtbs for the 5 groups, the control, dry cotton, wet cotton, water rinse, and ultrasonic rinse group, were 13.42 ± 4.68, 11.96 ± 5.26, 13.03±6.07, 12.73±3.68, and 14.39±4.22 MPa, respectively. No significant difference was found between the mean bond strengths of the groups (p=0.725). In SEM examination no residual sealer was found in any group.

**Conclusions:** Calcium silicate-based bioceramic sealer was removed from the dentin surface with all removal protocols when evaluated with Mtbs after thermal and mechanical cycle tests.

**Background**

Coronal restorations are very important for increasing the success of root canal treatment [1–3] as they prevents bacterial invasion and endotoxin infiltration from the coronal direction by creating an additional barrier to the canal orifice and occluding the pulp chamber [4].

Different modes of adhesive can be used in the restoration of the endodontic access cavity. One bottle of self-etch adhesive with relatively easy application and low technical precision is used for coronal sealing immediately after root canal filling [5].

The bond strength of adhesives is lower when the dentin surface is contaminated by hemostatic agents [6], zinc oxide eugenol-containing cements [7] or endodontic sealer residues [8–10]. Furthermore remnants can also cause tooth discoloration [11, 12], thereby creating aesthetic issues for the patient. Chemical solvents such as ethanol, acetone, amyl acetate or isopropyl alcohol should be used to remove root-canal sealer residues [13–15].
Bioceramic root canal sealers have entered dentistry in recent years and have been presented to the market with features such as osteoinductive effect [16], hardening of tissue fluids, long-term antibacterial effect [17–19], biocompatibility [20–23], long working time, and expansion in the root canal [24].

There are studies evaluating the effects of eugenol [8, 9] and resin-based root canal sealers [10] on dental adhesive systems and reporting negative outcomes. The bonding of calcium silicate-based bioceramic canal sealers to root dentin [25, 26] and gutta percha [27] was evaluated. However, it is not known how bioceramic pastes are removed and how they affect the adhesion of self-etch adhesives. The aim of this study is to evaluate the different removal techniques of calcium silicate-based canal sealers from endodontic access cavities.

The null hypothesis of this study is that cleaning contaminated dentin with a calcium silicate-based bioceramic canal sealer with different removal protocols does not cause the deterioration of the strength of the resin-dentin bonds created by self-etching adhesives.

**Methods**

Sixty extracted, erupted, noncarious, nonrestored, uncracked human lower third molars were gathered by the Non-Invasive Clinical Research Ethics Committee of Cukurova University Faculty of Medicine, dated 13.03.2020, numbered 42. Informed consent forms were signed by all patients. The procedures used in this study adhere to the tenets of the Declaration of Helsinki. Furthermore, teeth of nearly equal size and similar occlusal anatomy were selected.

Teeth were embedded in self-curing acrylic resin, and standardized uniform box-shaped Class I cavities (4 mm × 4 mm × 4 mm) were prepared with a high-speed handpiece under air-water spray using cylindrical flat-end diamond burs. The burs were replaced after every five preparations. Teeth were randomly divided into 5 groups according to the removal protocol (n = 10 per group). The remaining 10 teeth were used for scanning electron microscopy (SEM) analysis. The cavity of the control group (G1) was not contaminated with canal sealer. The teeth in the experimental groups were dried with an air stream. Approximately 1-mm-thick layer of calcium silicate-based Endosequence BC Sealer (Brasseler, Savannah, GA, USA) was injected on the surface of the dentin at the base of the cavity and left for 5 minutes. According to the removal protocol, uncured sealer remnants were removed from cavities as follows: G2- A dry cotton pallet, 2×size nr. 2, was used to scrub the access cavity walls for 5 seconds, the same process was repeated by new a dry cotton pallet, G3- A wet cotton pallet, size nr. 2, was used to scrub the access cavity walls for 5 seconds, the same process was repeated by new a wet cotton pallet, G4- Access cavity walls were rinsed with the air/water spray from a dental unit for 5 seconds, and G5- by passively applying aqueous ultrasonic energy without any contact with the canal walls for 5 seconds with ultrasonic scaler (PiezonMaster 400, EMS, Nyon, Switzerland).

After the removal of the canal sealer, the cavity surface was restored with a one-bottle universal adhesive (G-Premio BOND, GC, Tokyo, Japan) and in two 2-mm-thick vertical layer composite resins (G-ænial posterior, GC, Tokyo, Japan) according to the manufacturer's instructions. Each layer was cured for 20
seconds (Elipar Freelight, 3M-ESPE, Minn, USA). All the restored specimens were stored for 7 days at 37°C in distilled water to allow for complete sealer setting and were then subjected to both thermocycling and mechanical loading in a chewing simulator. Mechanical loading was performed with a 50 N load for 240,000 cycles. The load was vertically applied on the central fossa of the molar with a steel ball (6 mm in diameter) at a frequency of 1.6 Hz. A 0.5-mm sliding movement was also applied during loading. Thermocycling was performed for 1000 thermal cycles in deionized water from 5°C to 55°C, with a 60-second dwelling time and a 10-second transfer between temperature baths.

After simulating aging, the restored teeth were sectioned into three or four 1-mm-thick slices perpendicular to the bonded surface using a slowspeed diamond saw (Diamond cutt-off Wheel MOD15, Struers, Rødovre, Denmark) under water irrigation (Accutom 10, Struers, Rødovre, Denmark). The tooth was then rotated 90 degrees and sectioned again to obtain 1-mm² sticks. The cross-sectional area of each specimen was measured with a digital calliper (Shinwa, Osaka, Japan) and recorded to calculate the bond strength. Specimens that failed during sectioning were excluded. The beams were fixed to the jig by cyanoacrylate glue (Pattex, Henkel, Duesseldorf, Germany) and tested in a universal testing machine (MOD Dental MIC-101, Esetron Smart Robotechnologies, Ankara, Turkey) at a cross-head speed of 0.5 mm/min. The tensile strength was measured in Newtons. The µTBS values were calculated in megapascals (MPa) by dividing the fracture force by the cross-sectional surface area. Fractured specimens were then examined under a 40× magnification stereomicroscope (SOIF optical instruments, Stereo microscope ST6024-B2, Istanbul, Turkey) to analyze fracture mode.

For SEM analysis, 2 teeth in each experimental group were cut in half longitudinally in a buccolingual direction from the center of the restoration with a water-cooled diamond disc (Struers, Diamond cutt-off Wheel MOD15, Rødovre, Denmark). The specimens were dehydrated for 24 hours at room temperature and mounted on aluminum stubs, desiccated, sputter coated with gold/palladium, and examined under a scanning electron microscope (FEI, Quanta 650 FEG, Oregon, USA).

The Shapiro-Wilk test was used to evaluate the normality of the data. The normally distributed data were analyzed by one-way analysis of variance (ANOVA) and the Tukey HSD (IBM SPSS Statistics V21 Armonk, USA).

**Results**

The microtensile bond strength results are shown in Table 1. Seven specimens were excluded due to inadequate remaining dentin thickness (< 1 mm) (2) or the fracture during the removal of the sticks from the cavity (5). The mean bond strengths for the 5 groups, which were the G1: control group, G2: dry cotton, G3: wet cotton, G4: water rinse, and G5: ultrasonic rinse, were 13.42 ± 4.68, 11.96 ± 5.26, 13.03 ± 6.07, 12.73 ± 3.68, and 14.39 ± 4.22 MPa, respectively. No significant difference was found between the mean bond strengths of the groups (p = 0.725). The highest bond strength was in the ultrasonic rinse group, followed by the control, wet cotton wiping, water rinse and dry cotton wiping groups.
Table 1
Microtensile Bond Strength (MPa) for All Specimen Groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dry Coton</th>
<th>Wet Coton</th>
<th>Water rinse</th>
<th>Ultrasonic rinse</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>38</td>
<td>38</td>
<td>39</td>
<td>40</td>
<td>38</td>
</tr>
<tr>
<td>Mean</td>
<td>13,42a</td>
<td>11,96a</td>
<td>13,03a</td>
<td>12,73a</td>
<td>14,39a</td>
</tr>
<tr>
<td>SD</td>
<td>4,68</td>
<td>5,26</td>
<td>6,07</td>
<td>3,68</td>
<td>4,22</td>
</tr>
</tbody>
</table>

The μTBS values are expressed as means and standard deviation. Groups with the same letters are not statistically different (p > 0.05).

Adhesive Failure

Greater adhesive failures were observed in all groups. Groups presented similar percentages of fracture patterns (Table 2).

Table 2
Fracture patterns of bonded specimens after bond strength test. %

<table>
<thead>
<tr>
<th></th>
<th>Adhesive</th>
<th>Cohesive</th>
<th>Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>83</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Dry coton</td>
<td>94</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Wet coton</td>
<td>88</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Water rinse</td>
<td>85</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Ultrasonic rinse</td>
<td>78</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

SEM Evaluation

In the samples examined at 5000× magnification, no residual sealer was found in any group (Fig. 1, 2). All of the cleaning protocols were able to completely remove Endosequence BC Sealer from the dentin surface.

Discussion

The sealers used during root canal obturation contaminate the floor and walls of the endodontic access cavity. This contamination has to be removed for successful and long-term dentin restoration bonding. In the present study, removal protocols for contamination caused by calcium silicate-based endosequence
BC sealer were evaluated. The sealer was removed by all removal methods using water, including dry cotton. Thus, the null hypothesis is not rejected.

Calcium silicate-based bioceramic sealers produce calcium hydroxide by hydration, which affects water sorption and solubility [28]. Therefore, we used water as the solvent to remove the residual calcium silicate sealers in the study. In our study, the highest bond strength was measured in group 4, in which water was activated by ultrasonication. The lowest bond strength was measured on cavity surfaces cleaned with dry cotton. The ultrasonic activation of the water resulted in higher bond strength compared to the control group, as it allowed proper removal of residual sealer and even smear layer and debris. In SEM images of the dentin-adhesive interface showed a thin hybridized layer and few/short resin tags created by the self-etch adhesive. No residual sealer was observed in any of the specimens.

The most commonly used method to characterize the durability of adhesives is in vitro bond strength tests. Microtensile test results are influenced by many physical and chemical factors, i.e., variations in dentin depth, tubule diameter, cavity configuration [29, 30], and mechanical and thermal stresses [31, 32].

The microtensile bond strength of the G-premio bond in flat dentin without aging is approximately 25–35 MPa [33, 34]. In the current study, the mean of microtensile bond strength of the G-premio bond was measured to be 11.96(± 5.26)-13.03(± 6.07) MPa. The reason for this decrease may be the aging process and the use of cavity dentin. High percentage of adhesive failure in all groups may be the result of aging.

Microtensile samples prepared with flat dentin do not imitate the clinical situation. Studies have shown that “flat” versus “cavity” dentin influences microtensile bond strength due to differences in the configuration (“C”) factor [35, 36]. In our study, the root canal was reached by opening box-shaped class I cavities to the lower molar teeth to mimic the clinical access cavity design.

In our study, the dry cotton group had less bond strength than the water-used groups, but no statistically significant difference was found. The reason for this may be that the polymerization shrinkage due to the C factor and the stresses created by the mechanical loads exceeded the removal advantage. The cavity configuration factor is the ratio of the bonded surface area to the unbonded surface area. This means that in a box-like class I cavity, 5 times more polymerization shrinkage stress is exposed than flat dentin.

Epoxy resin-based sealers adversely interfere with the bond of dentin adhesives [9], because resin monomers of sealer and adhesive are chemically different so they do not copolymerize. Therefore several solutions containing ethanol, ethyl acetate, acetone, or chlorhexidine digluconate have been recommended for removal of epoxy resin based sealer residues from the dentin surface [37]. However, the effective removal of calcium silicate-based cements with all protocols may be seen as an advantage for clinical use. Another advantage of using the bioceramic sealer does not shrink upon setting and creates strong chemical bonding with dentin [38].

Studies have reported immediately high in vitro bond strength. To determine the long-term bond strength of adhesives, the value after exposure to chewing forces and temperature changes should be measured.
Thermal cycling may produce hydrolytic degradation, which changes the stress/strain levels transferred to the interface of two materials and reducing the bond strength [39]. Also mastication causes stress in the restored dentin system. Studies have shown that cyclic loading significantly reduces the bond strength of the adhesive [31, 40]. The maximum bite force can be increased from 200 N to 360 N in the posterior area [41]. During the biting process, activation in the muscles is in the frequency range of 1.5–1.8 Hz for each half cycle [42]. According to the literature, approximately 240,000–250,000 cycles in the chewing simulator correspond to yearly clinical use [43]. In our study, we subjected the restoration to a chewing simulator of 240,000 cycles at a frequency of 1.6 Hz with a force of 50 N in the horizontal and vertical directions for 1 year of aging.

Bond strength measurements are appropriate methods to assess the coronal seal. Within the limits of this ex vivo study, it can be concluded that all removal protocols are effective on the resin-dentin bond strengths of the self-etching adhesive. Further clinical trials and research must be performed to evaluate the removal protocols of calcium silicate-based sealers.

**Conclusion**

According to results of this study, calcium silicate sealer was removed from dentin surface with all clinical techniques. All removal protocols showed the same results when evaluated with µTBS after thermal and mechanical cycling tests.

Another advantage of using bioceramic sealer not having shrinkage and creates strong chemical bonding to dentin

**Declarations**

**Ethics approval and consent to participate**

The protocol was reviewed and approved by Non-Invasive Clinical Research Ethics Committee of Çukurova University Faculty of Medicine (No: 13-03-20-/42).

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

**Competing interests**

The authors declare there have no competing interests.
Funding

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Authors’ contributions

ZGBK developed the study outlines and coordinated the protocol. ZGBK collected the data; ZGBK and OY analyzed the data and OY contributed to revision of the final draft. All authors read and approved the final manuscript.

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References


**Figures**

![Figure 1](image)

**Figure 1**

SEM image of resin dentin interface. (D: Dentin, A: Adhesive, C: Composite resin, RT: Resin tag)
Figure 2

SEM image of resin dentin interface. (D: Dentin, A: Adhesive, C: Composite resin, RT: Resin tag)