The Effect of Recommended Mouthwashes on the Shear Bond Strength of Orthodontic Brackets During the Covid-19 Pandemic: An In Vitro Study

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Article Info

ABSTRACT

Objectives: This study aimed to assess the impact of recommended mouthwashes (chlorhexidine, hydrogen peroxide, and povidone-iodine) used during the COVID-19 pandemic on the shear bond strength (SBS) of orthodontic brackets.

Materials and Methods: A total of 52 human premolar teeth were divided into 4 groups (N=13) consisting of no intervention (control group), 0.2% chlorhexidine, 1.5% hydrogen peroxide, and 0.2% povidone-iodine. Following immersion in the mouthwashes, orthodontic brackets were bonded to enamel surfaces. Then, the brackets were debonded using a universal testing machine. The specimens were evaluated with a scanning electron microscope (SEM) and adhesive remnant index (ARI) score was assessed. One-way ANOVA and Tukey test were used for statistical analysis and P<0.05 was considered significant.

Results: The SBS of the experimental groups decreased compared to the control group. The highest SBS mean value was observed in the control and the lowest was found in the 0.2% povidone-iodine group. Significant differences in SBS values were found between the 0.2% povidone-iodine group and the 1.5% hydrogen peroxide group when compared to the control group (P=0.023, P=0.028, respectively). SEM analysis revealed similar characteristics among the groups, with a closer resemblance between the chlorhexidine and control groups. Additionally, these groups exhibited greater etching depth compared to the other groups.

Conclusion: Our findings highlight the importance of careful selection and application of mouthwashes in orthodontic procedures. While 1.5% hydrogen peroxide and 0.2% povidone-iodine may have some impact on bond strength, their use can still be considered acceptable within defined limits.

Keywords: Mouthwashes; Orthodontic Appliances; Povidone-iodine; Hydrogen peroxide; Chlorhexidine; Anti-Infective Agents; COVID-19

INTRODUCTION

There is a risk of virus transmission through the oral cavity, oropharynx, and nasopharynx due to the high viral load found in the saliva and respiratory tract [1,2]. As a result, many professional associations and organizations have developed guidelines and advice on preventing the transmission of SARS-CoV-2 from patients to dentists. Several associations recommend the use of different pre-procedure mouthwashes to reduce the viral load in the mouth and the aerosols that originate during dental procedures [3,4]. Between various types of mouthwash, using 0.12-0.2%, chlorohexidine 1.5% hydrogen
peroxide, and 0.2% povidone-iodine for 30 to 60s can reduce the amount of virus and microbial load in the oral cavity [5-9]. Chlorhexidine mouthwash has an immediate and short-lived bactericidal effect followed by a long-term bacteriostatic effect, which is dependent on the antiseptic absorbed by the pellicle coating tooth surfaces [10]. Chlorhexidine increases cell wall permeability, causing lysis of gram-positive and gram-negative bacteria, aerobes, anaerobes, and fungi [11]. Studies have shown that the coronavirus is sensitive to the activities of free radical oxygen resulting from hydrogen peroxide, which deactivates the virus in vitro and in vivo [12,13]. Povidone-iodine mouthwash executes its antimicrobial effect by separating the free iodine from polyvinyl pyrrolidone; this iodine penetrates microbes and destroys their proteins and nucleic acid structures, resulting in their death [14,15].

Based on the literature, there are limited data on the effect of povidone-iodine and hydrogen peroxide mouthwashes on shear bond strength (SBS) of orthodontic bracket to enamel; there also are limited studies on the chlorhexidine mouthwash on SBS to the enamel before bonding the bracket. Even though using pre-bonding mouthwashes decreased infection transmission, it could also influence the bond strength adversely. Therefore, the purpose of this study was to compare the effect of different mouthwashes that have been recommended during the Covid-19 pandemic (chlorhexidine, hydrogen peroxide, and povidone-iodine) on the shear bond strength of the orthodontic bracket.

**MATERIALS AND METHODS**

**Sample size calculation:** Based on a study by Demir et al [16] and considering \( \alpha=0.05, \beta=0.2, \) standard deviation=5.9, and effect size=0.43, the sample size was calculated by the one-way ANOVA power analysis option in SPSS and was determined to be 13 samples in each group.

**Ethics statement:** All experiments have been approved by the Ethics Committee of Tehran University of Medical Sciences (IRTUMS.DENTISTRY.REC.1399.260).

**Sample preparation:** A total of 52 premolars extracted for orthodontic purposes over the past six months were collected for this study. The teeth were deposited in a 0.5% chloramine solution during this period. Initially, the preservation solution was replaced every 24 h and then every month. Teeth were examined with a stereomicroscope (SMZ800, Nikon, Japan) at \( \times 10 \) magnification. The teeth exhibited normal anatomy and intact enamel on the buccal surface, with no signs of hypomineralization, caries, fractures, or restoration. Then, they were randomly divided into four groups:

1. Control group
2. 0.2% Chlorhexidine (Vi-one, Lacer Health Company, Spain)
3. 1.5% Hydrogen peroxide (Colgate, Manhattan, New York)
4. 0.2% Povidone-iodine (prepared from commercially available solution)

**Bonding procedure:** Orthodontic metal brackets (American Orthodontics, Sheboynan, WI, USA) were used in this study. The brackets were bonded to the teeth according to one of the following methods:

**Control group:** Teeth were etched with 37% phosphoric acid gel (3M, Dental products, St.Pou) and bonded with the etch-and-rinse composite (Transbond XT, 3M Unitek, Monrovia, California) following the manufacturer’s instructions.

**Experimental groups:** the samples were immersed in mouthwash for 60 seconds. Then rinsed with water for 10 seconds, and the bonding procedure was done according to the control group.

In all groups, once the brackets were firmly pressed onto the sample surfaces to ensure even distribution of adhesive resin under-neath, any excess resin was meticulously removed. The curing process was carried out using a light-emitting diode device (LED D Curing Light, Guilin Woodpecker, China 1400mW/cm²) for a total of 40s, evenly distributed with 10s of exposure from each side. After bonding, the samples were stored in distilled water (Morvarid pars, Tehran, Iran) at 37°C for 24h to prepare for thermocycling.

**Thermocycling:** The specimens underwent 5000 thermal cycles,
alternating between temperatures of 5°C and 55°C, while immersed in a water bath. Each cycle lasted for 20 seconds, with a 10-second pause at each temperature extreme, using a thermocycling machine (TC300, Vafaei Industrial, Iran) [17].

Shear bond strength (SBS):
A steel rod with one flattened end was attached to the crosshead of a universal test machine (UTM, Zwick GmbH & Co, Ulm, Germany). An occlusogingival load was applied to the bracket base, producing a shear force at the bracket-tooth interface. A computer electronically connected with the UTM recorded the results of each test. SBS were measured at a crosshead speed of 0.5mm/minute.

The highest applied forces were measured in Newtons (N) and then converted into SBS values expressed in megapascals (MPa). This conversion was achieved by dividing the recorded forces by the accurately determined cross-sectional area of the bracket, which was measured using an electronic gauge (10.28 mm²).

Adhesive remnant index (ARI):
All samples were examined under a stereomicroscope (Nikon D-CS, Japan) with a magnification of ×10. Scoring was done according to previous studies [17,18]:
- Score 0: less than 10% of the adhesive remains on the tooth.
- Score 1: 10%–50% adhesive remains on the tooth.
- Score 2: 50%–90% adhesive remains on the tooth.
- Score 3: more than 90% of the adhesive remains on the tooth (with visible mesh pattern).

Scanning electron microscope (SEM) evaluation:
Two samples from each group (etched and non-etched) were selected for SEM analysis. The images were captured through SEM (S4160, Hitachi, Japan) to examine structural and morphological alterations on the tooth enamel surface, including the presence of porosity or irregularities. The micro-morphology of representative surfaces was obtained at magnifications of ×500, ×1500, and ×5000. All SEM analyses were conducted by an individual blinded to the mouthwashes used in this study.

Failure mode analysis:
A stereomicroscope was used for failure mode analysis at ×15 magnification. It was recorded as follows:
- "Adhesive failure": the fracture occurred along the junction of the adhesive and the enamel.
- "Cohesive" failure: if the fracture occurred in the adhesive or enamel.
- "Mixed" failure: fractures occurred in adhesive and enamel or adhesive margin.

The failure mode analysis was conducted by an operator blinded to the study groups.

Statistical analysis:
The maximum, minimum, mean, and standard deviation of each group were calculated. One-way analysis of variance (ANOVA) and Post-hoc Tukey tests were performed for SBS and chi-square test for ARI. The level of significance was set at P<0.05.

RESULTS
Shear Bond Strength (SBS):
Descriptive statistics for the SBS values of all groups are shown in Table 1. Analysis of variance indicated that the SBS of the experimental groups were lower than the control group. As shown in Figure 1, when compared to the control group, the 1.5% hydrogen peroxide and the 0.2% povidone-iodine groups had significantly lower SBS (P=0.028 and P=0.023, respectively). There was no significant difference in SBS among the experimental groups (P>0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>SD</th>
<th>SE</th>
<th>95% CI for the Mean</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>21.35</td>
<td>14.71</td>
<td>29.72</td>
<td>4.61</td>
<td>1.28</td>
<td>18.57</td>
</tr>
<tr>
<td>0.2% Chlorhexidine</td>
<td>18.84</td>
<td>12.37</td>
<td>36.72</td>
<td>6.21</td>
<td>1.72</td>
<td>15.09</td>
</tr>
<tr>
<td>1.5% Hydrogen peroxide</td>
<td>15.5</td>
<td>11.10</td>
<td>23.9</td>
<td>4.19</td>
<td>1.16</td>
<td>12.96</td>
</tr>
<tr>
<td>0.2% Povidone-iodine</td>
<td>15.36</td>
<td>8.92</td>
<td>23.56</td>
<td>5.34</td>
<td>1.48</td>
<td>12.13</td>
</tr>
</tbody>
</table>

Min: minimum; Max: maximum; SD: standard deviation; SE: standard error; CI: confidence interval.
Fig. 1. Comparison of shear bond strength of brackets on enamel treated with various mouthwashes (*statistically significant with P<0.05)

**Adhesive Remnant Index**

Table 2 shows the distribution frequency of the ARI score for each group. Generally, the amount of adhesive remnant in the mouthwash groups was less than the control groups. The type of failure was mostly scored 0 and 1 in all groups. In the control group, the lowest failure type was scored 3 (7.7%). In the 0.2% chlorhexidine group, score 0 (38.5%) increased by 8.5% compared to the control group (30.8%) and was the most common failure type. In the 0.2% povidone-iodine group, score 0 types of failure (69.2%) increased by 38.4% compared to the control group (30.8%) and were the most common failure type. In the 1.5% hydrogen peroxide group, score 0 types of failure (61.5%) increased by 30.7% compared to the control group (30.8%) and were the most common failure type.

**Failure mode analysis:**

Table 3 presents the failure mode analysis frequency distribution of all tested groups. The predominant failure mode was the adhesive type for povidone-iodine 0.2% and hydrogen peroxide 1.5% groups. For the chlorohexidine 0.2% and the control groups, the most frequently observed failure mode was the cohesive type.

**Table 2.** Frequency distribution of the adhesive remnant index in the study groups (N=13)

| Groups | 0 | 1 | 2 | 3 |  
|--------|---|---|---|---|-----|
| Control| 4 | 30.8 | 4 | 30.8 | 4 | 30.8 | 1 | 7.7 |
| 0.2% CHX | 5 | 38.5 | 4 | 30.8 | 4 | 30.8 | 0 | 0 |
| 1.5% HP | 8 | 61.5 | 4 | 30.8 | 1 | 7.7 | 0 | 0 |
| 0.2% PI | 9 | 69.2 | 3 | 23.1 | 1 | 7.7 | 0 | 0 |

CHX: Chlorhexidine; HP: hydrogen peroxide; PI: povidone iodine

**Table 3.** Frequency distribution of the location of the failure in the study groups (N=13)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Location of the failure</th>
<th>N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td>Control</td>
<td>4 (30.8)</td>
<td>8 (61.5)</td>
</tr>
<tr>
<td>0.2% CHX</td>
<td>2 (15.4)</td>
<td>8 (61.5)</td>
</tr>
<tr>
<td>1.5% HP</td>
<td>6 (46.2)</td>
<td>5 (38.5)</td>
</tr>
<tr>
<td>0.2% PI</td>
<td>9 (69.3)</td>
<td>3 (23.1)</td>
</tr>
</tbody>
</table>

CHX: Chlorhexidine; HP: hydrogen peroxide; PI: povidone iodine
**Tooth surface evaluation:**
Figure 2 displays the SEM images of the tooth enamel surface before and after etching in the control group. The images clearly depict a honeycomb pattern at various magnifications. Figures 3, 4, and 5 respectively show the surfaces of the enamels that were stored in chlorohexidine, hydrogen peroxide, and povidone-iodine mouthwashes before and after etching with different magnifications.

Fig. 2. Group 1, scanning electron microscopic images of the enamel surface in the control group before (A1, A2, A3) and after etching (B1, B2, B3) at ×500, ×1500 and ×5000 magnification. The honeycomb structure and porosity are visible on the surface of the etched enamel.

Fig. 3. Group 2, scanning electron microscopic images of the enamel surface of the chlorhexidine group before (A1, A2, A3) and after etching (B1, B2, B3) at ×500, ×1500 and ×5000 magnification. The honeycomb structure and porosity are visible on the surface of the etched enamel.
Fig. 4. Group 3, scanning electron microscopic images of the enamel surface of the hydrogen peroxide group before (A1, A2, A3) and after etching (B1, B2, B3) at ×500, ×1500 and ×5000 magnifications. In addition to the honeycomb structure, a limited number of shallow porosities are visible on the etched enamel surface.

Fig. 5. Group 4, scanning electron microscopic images of the enamel surface of the povidone-iodine group before (A1, A2, A3) and after etching (B1, B2, B3) at ×500, ×1500 and ×5000 magnifications. The honeycomb structure and a few shallow porosities are visible on the etched enamel surface.

DISCUSSION
The virus in the saliva of COVID-19 patients can be transmitted to dentists, dental team members, and their patients [19]. Various types of mouthwash have been recommended to reduce the viral load; in previous studies, hydrogen peroxide, chlorhexidine, and povidone-iodine were commonly recommended during the COVID-19 pandemic (9). Preoperative mouthwashes may affect bond
strength to enamel; studies that examined the effects of recommended preoperative mouthwashes on bond strength to enamel evaluated dental universal adhesive systems. The results indicated that preoperative mouthwashes (chlorhexidine, hydrogen peroxide, and povidone-iodine) decreased bond strength to enamel; hydrogen peroxide and povidone-iodine significantly decreased shear bond strength in both self-etch and etch-and-rinse groups [20,21].

Orthodontic brackets also employ bonding adhesives. A bond with over-optimistic strength will damage the tooth enamel during debonding at the end of treatment, whereas a bond with under-optimistic strength will cause the bracket bond to the enamel to fail continuously during treatment; thus, the bond must be strong to avoid experiencing these issues. In addition, many factors influence bracket bonding strength in fixed orthodontic treatments [22]. Studies have shown that some substances (e.g., acidic beverages, herbal teas, acidic and alcoholic foods, and chemical solvents) affect the SBS of orthodontic bracket [23-26].

There are limited data regarding the effect of recently recommended pre-procedural mouthwashes on the bond strength of orthodontic brackets to the enamel. Consequently, this in vitro study compared the efficacy of various preoperative types of mouthwash recommended during the COVID-19 pandemic to reduce the viral load on the SBS of the orthodontic brackets.

The results of this study showed that mouthwashes decreased the SBS; this decrease was statistically significant in povidone-iodine 0.2% and hydrogen peroxide 1.5% groups. Nevertheless, this decrease was not statistically significant for chlorhexidine 0.2%. Therefore, povidone-iodine 0.2% and hydrogen peroxide 1.5% had adverse effects on orthodontic therapy; based on the clinically acceptable range (6-8 MPa), the bond strength of the study groups was almost within this range, and allowing them to be used as a treatment in the clinic [27]. The result of Filler et al [28] was in line with our study and showed that there was no significant difference between SBS for the control and experimental groups (0.12% chlorhexidine, one minute, four times daily, for seven days). Also, the results of this study indicated that the use of antibacterial rinse would not compromise composite bond strength; they did not evaluate the effect of hydrogen peroxide and povidone-iodine on bond strength.

The result of the current study agreed with the study of Özduaman et al, [20] in which they evaluated the effects of pre-operative mouthwashes on bond strength to enamel and reported that SBS of pre-procedural oral rinse with hydrogen peroxide 1.5% and povidone-iodine 0.2% are significantly lower than another group (control and chlorhexidine 0.2%). But the results of this study were contrary to the other study; According to Demir et al [16], the application of chlorhexidine (0.2%, 15s) and povidone-iodine (7.5%, 15s) before acid etching did not reduce bond strength; the authors considered that this may be due to either the lack of effect of chlorhexidine or acid etch that dissolves the enamel before bonding.

One of the mechanisms that could interfere with the adhesive strength was free oxygen [29, 30]; this free radical prevents resin polymerization, and as a result, it can disrupt their bonding strength. Rego et al [31] examined the effects of hydrogen peroxide 35% on the SBS and found that hydrogen peroxide 35% reduced bond strength within 24 hours. However, after seven days, it did not significantly differ from the control group. So far, studies have been conducted on the effects of hydrogen peroxide as a bleaching material on the bond strength of orthodontic composites. No research has been done about the impact of free radicals produced by mouthwashes on the bonding strength. However, it should be considered as a factor that reduces the bond strength of the orthodontics composites. It can prevent the polymerization of the composite; hence, it affects the morphology of the tooth enamel. Some studies had demonstrated that protein and minerals of the surface layers of the tooth enamel change by free radicals, and this process reduces the bond strength [32].

In the present study, by investigating the SEM of the samples, the surface changes made by these mouthwashes, considering the short period of
their use did not make a significant change in the enamel structure; only the lesser amount of the etching in two hydrogen peroxide and povidone-iodine.

Although the current study had the advantage of examining the effect of pre-operational mouthwashes on orthodontic bracket bond strength, the major limitation of our study was the difficulty to compare an in vitro study with the conditions in clinical practice. It would be beneficial to conduct further studies with different bonding systems, such as glass-ionomer or adhesive resin, different concentrations of mouthwashes, and different adhesive systems (self-etch and etch-and-rinse methods).

**CONCLUSION**

The results of this study can be summarized as follows:

- 1.5% Hydrogen peroxide and 0.2% povidone-iodine significantly decreased the shear bond strength of orthodontic composites but the reduction was within acceptable limits.
- Analysis of the AIR index showed that the mode of failure across all groups was primarily scored as 0 or 1.
- The predominant failure mode in the 0.2% povidone-iodine and 1.5% hydrogen peroxide groups was of the adhesive type and in the 0.2% chlorohexidine and the control groups was of the cohesive type.
- SEM observations highlighted that the etching pattern of the control group and chlorohexidine 0.2% group was similar; but, in the 0.2% povidone-iodine and 1.5% hydrogen peroxide groups, the etching depth and porosity were less than the chlorohexidine and control groups.

**CONFLICT OF INTEREST STATEMENT**

None declared.

**REFERENCES**


