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Original Article

Orthodontic brackets' shear bond strengths after applying remineralizing agents

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Abstract

Aim: The aim of this in vitro study was to measure the effects of white spot lesions treatment agents on the shear bond strengths (SBSs) and adhesive residual indexes (ARIs) of orthodontic brackets.

Methodology: This study used 100 human premolar teeth randomly divided into five groups. Group 1 comprised those with intact enamel, Group 2 comprised those with demineralized enamel and Groups 3-5 comprised those demineralized enamel that was treated with casein phosphopeptide amorphous calcium phosphate fluoride (CPP-ACPF), fluoride varnish and a resin infiltrant, respectively. Brackets were bonded to the teeth using the conventional method, then the samples were thermocycled and tested for SBS using a universal testing machine. The adhesive remnant indexes (ARI) of the brackets were also evaluated. One-way ANOVA and post-hoc Tukey tests were used to compare the groups' SBSs and the Kruskal-Wallis test was used to evaluate the groups' ARI scores. Results were considered statistically significant if p was less than 0.05.

Results: Statistically significant differences were found between the groups (F was 6.895 and p was less than 0.001). The SBSs of the brackets in Group 4 were significantly lower than those of the other groups (the mean was 13.44 ± 6.37 MPa). Group 5 had the highest mean SBS value (22.11 ± 6.56 MPa). Additionally, the ARI scores of the four groups were significantly different (p was less than 0.001).

Conclusion: Resin infiltration and CPP-ACPF applications can improve bonds to demineralized enamel, while fluoride varnish applications are not recommended for such enamel.

Keywords: CPP-ACPF, shear bond strength, demineralization, resin infiltration, brackets

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Introduction

Dental caries is a disease that affects the hard tissues of the teeth and occurs with a multifactorial aetiology. Caries formation starts from the outermost part of the tooth, the enamel layer. Enamel caries is initially opaque, white in colour and is called a white spot lesion (WSL). WSL is defined as the subsurface porosity of demineralized carious enamel localised on a flat surface, manifesting as milky white/opaque colouration (1). These lesions usually occur as a result of disruption of the demineralization-remineralization cycle on the enamel surface as a result of an acidic

environment in the presence of inadequate oral hygiene. Although WSLs re-mineralize over time, the opaque appearance of the tooth does not change and causes an unaesthetic look (2, 3).

Patients receiving fixed orthodontic treatment, who cannot provide ideal oral hygiene, are at high risk of increased WSLs (4). The areas of clinically determined enamel decalcifications are places where bacteria remain for a long time and are difficult to clean. WSLs occur in these regions from increased demineralization and because plaque prevents re-mineralization (5, 6). Orthodontic bands, brackets or more complex treatment options create a retaining area for plaque in the mouth and prevent muscle and salivary activities that play a role in the natural removal of these attached plaques (7). In this situation, low-pH plaque containing fermented carbohydrates forms. The setting also increases the colonisation of acidic bacteria such as Streptococcus mutans and lactobacilli (6-8).

The prevalence of a WSL after orthodontic treatment varies depending on the measurement method, criteria and inclusion of pre-existing developmental enamel defects in the assessment (9). Studies have reported a WSL prevalence of 2% to 96% (6, 8). Depending on the duration of orthodontic treatment, the incidence and severity of WSLs in the mouth may differ. WSL can occur in a period of 4 weeks, which is equivalent to the time between two sessions of orthodontic treatment (10).

Currently, the efficacy of several different methods in the treatment of WSL has been investigated (11, 12). Great interest surrounds the noninvasive treatment of WSLs, which focus on the use of topical fluoride agents associated with diet and good oral hygiene to promote lesion remineralization (13). Remineralization, which is the natural repair process for noncavitated lesions, relies on fluoride-supplemented calcium and phosphate ions to form a new surface on existing crystal residues in subsurface lesions remaining after demineralization (14). Fluoride ions are incorporated into remineralized enamel/dentin, converting carbonated apatite into a more acid-resistant, fluoroapatite-like form that confers additional acid resistance on hard tissues (15). One study showed that the use of fluoride varnish in patients undergoing orthodontic treatment has reduced enamel demineralization by 43.3% (16).

Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) is a nanocomplex of milk protein (casein phosphopeptide, or CPP) and amorphous calcium phosphate (or ACP) (17). CPP can form ACP nanoclusters by binding calcium and phosphate ions. These CPP-stabilised ACP nanoclusters can retain high concentration gradients of calcium and phosphate ions and ion pairs within the subsurface lesion. The increased ion concentration in the lesion fluid results in the formation of hydroxyapatite or fluoroapatitet via crystal growth, thereby suppressing enamel demineralization and increasing remineralization (18, 19). When sufficient levels of calcium and phosphate ions are combined with fluoride ions (CPP-ACPF), significant remineralization of enamel lesions results. Fluoride combined with CPP-ACP incorporates into the body of the WSL and is not localised to the outermost surface layer of enamel. Diffusion of fluoride ions along with calcium and phosphate ions deep into the lesion allows significant crystal growth (remineralization) throughout the body of the lesion (19, 20).

To stop incipient caries lesions, preventive and reparative treatments can be replaced by low-viscosity, light-cured resins to reach subsurface lesions (21). In randomised, controlled clinical studies, such an approach has effectively stopped flat-surface enamel lesions. Instance infiltration concept (ICON) is a type of resin infiltration (22, 23).

The incidence of WSLs in individuals who have not received orthodontic treatment reportedly ranges from 11% to 24%, so numerous patients have WSLs before orthodontic treatment (6, 24, 25). The likelihood of developing WSLs may increase with the placement of fixed orthodontic appliances. Because of the high prevalence of WSLs during orthodontic treatment, appropriate agents on demineralized enamel must be used to stop WSL formation. These agents should have minimal negative effects on the bonding of the brackets to the enamel surface. The aim of this in vitro study was to measure the effect of the application of agents used for WSL treatment before orthodontic treatment on the bracket shear bond strength (SBS) and the adhesive residual index (ARI). The study consisted of five groups: the SBS and ARI control groups, a demineralized enamel surface group, a CPP-ACPF group and a fluoride varnish and resin infiltrated (ICON) group.

Materials and Methods

This in vitro study protocol was approved by the local ethical committee of the Aydın Adnan Menderes University, Faculty of Dentistry (ADÜDHF2021/021). G*power 3.1.9.2, a sample size calculation programme was used; with a group ratio of 1:1, the number of samples with an effect size of 0.40 was calculated as 18 in each group and a total of 90 for five groups. To account for possible data losses, the study enrolled 20 samples in each group, for a total of 100 samples. The type 1 error rate was 0.05, and the study power was 0.85.

Therefore, 100 maxillary premolar teeth extracted for orthodontic treatment were selected for the study. Samples had to meet the following inclusion criteria: no caries, no fillings, no developmental defects and no broken or cracked teeth. Selected teeth were stored in a 0.1% concentration thymol solution (maximum time, 1 month); they were cleaned under running water with a periodontal curette and then polished with fluoride-free pumice and rubber cups. The teeth were cut 3 mm below the enamel-cementum boundary with the help of a micromotor and diamond separator; the root parts were removed, and the crown parts were used. The crowns of the teeth were embedded in autopolymerising cold acrylic in standard-size PVC moulds with the buccal enamel surfaces on top and exposed. The teeth were randomly divided into five

equal groups as follows, including 20 samples in each group: Group 1 (G1): intact enamel as the control group; Group 2 (G2): demineralized enamel group; Group 3 (G3): demineralized enamel pretreated with CPP-ACPF; Group 4 (G4): demineralized enamel pretreated with 5% fluoride varnish; and Group 5 (G5): demineralized enamel pretreated with ICON.

Demineralization procedure

This study used the demineralization protocol described by Ekizer et al. (26). In this protocol, the teeth were immersed in a 40-mL solution with a pH of 4.3, containing 2.0 mmol/L of calcium, 2.0 mmol/L of phosphate and 75 mmol/L acetate. The solution was maintained for 6 h at 37°C for demineralization. Then, all teeth were treated in a 20-mL remineralization solution with a pH of 7.0, containing 1.5 mmol/L of calcium, 0.9 mmol/L of phosphate, 150 mmol/L of potassium chloride and 20 mmol/L of cocodylate buffer. This solution was maintained at 37℃ for 18 h. Before transfer from the demineralization tank to the remineralization tank, the teeth were passed through distiled water one by one. This cycle lasted 21 days.

Application of pretreatment agents

MI Paste Plus™ with Recaldent™ (GC Cooperation, Tokyo, Japan) was applied to 20 teeth in G3 after the demineralization procedure. After being applied to all samples for 5 min, the paste was removed from the tooth with cotton pellets; the samples were placed in an artificial saliva solution and kept in an oven at 37°C for 24 h. This cycle continued for 1 week.

Voco Pro Fluorid Varnish (Voco Gmbh, Cuxhaven, Germany) was used on 20 teeth in G4 after the demineralization procedure. The varnish was applied to all samples for 5 min and then removed with distiled water.

After the demineralization procedure of 20 teeth in G5, ICON (DMG, Hamburg, Germany) was used for the resin infiltration method. In accordance with the manufacturer's directive, Icon-Etch (HCl 15%) was applied to the lesion surface for 2 min, and then lesions were washed with water for 30 sec. Then, Icon-Dry (ethanol) was applied and dried with air spray for 30 sec. After the lesion opacity disappeared, Icon-Infiltrant was applied and left on the lesion for 3 min. The applied infiltrant was distributed on the tooth surface with the help of air and was irradiated with an LED light-curing device (Elipar™ S10 Curing Light; 3M, Monrovia, CA) for 40 sec.

Bonding procedure

Premolar brackets (0.22 slot; American Orthodontics, Sheboygan, WI) were attached in the conventional method to the middle of the buccal surface of the teeth in all groups. A 37% phosphoric acid gel (Reliance Orthodontic Products, Itasca, IL) was applied for 15 sec to the area where the brackets attached to the teeth; then, the teeth were washed with water for 15 sec and dried with oil-free air for 10 sec. After the drying process was completed, a thin layer of Transbond XT (3M Unitek, Monrovia, CA, USA) primer was applied to the tooth surfaces and left unpolymerised according to the manufacturer's instructions. Then, using Transbond XT (3M Unitek, Monrovia, CA, USA) composite resin, the residual adhesives around the brackets were cleaned, and light was applied to the teeth in the mesial and distal directions (10 sec in each direction, for a total of 20 sec) with the Elipar™ S10 device. All samples were placed in an artificial saliva solution and kept in an oven at 37°C for 1 week before bracket bonding and for 24 h after bracket bonding to complete the composite polymerisation process.

SBS test

Before the orthodontic SBS test, all samples were subjected to thermal cycling. In the thermal cycle, all samples were submerged in two different bodies of distiled water (at 5℃ and 55℃) for 30 sec, and the cycle was repeated 5000 times. The samples were removed from the water for 15 sec between submersions. The SBS of the samples was tested with a universal testing machine (MOD Dental; Esetron Smart Robotechnologies, Ankara, Turkey); the force values at the moment of rupture in the bracket-enamel connection were recorded in Newtons (N). The base surface area of the orthodontic brackets (10.4 mm^2) was measured and recorded with a digital caliper. Statistical analysis of the data was reported in megapascals (MPa; 1 MPa = 1 N/mm^2) and was calculated by dividing the force value by the base area of the bracket. The head speed of the device was adjusted to 1 mm/min and a load cell of 2.5 kg.

ARI

After the brackets were debonded, the enamel surface of each tooth was examined under a stereomicroscope (Olympus SZ61, Munster, Germany) at 4.5× magnification to evaluate the ARI. Årtun and Bergland (27) defined a five-stage scoring system, as follows: Score 0: no residue of adhesive on tooth surface; Score 1: less than 50% of the tooth surface has residual adhesive; Score 2: more than 50% of the tooth surface has residual adhesive; Score 3: all tooth surface is covered with adhesive; and Score 4: enamel crack exists.

Statistical analysis

Analysis of the data was conducted in the IBM SPSS Statistics version 22 (IBM Corporation, Armonk, NY, US) package program. The Shapiro-Wilk normality test and Levene's variance homogeneity tests were applied to the data. The data distribution was normal; thus, one-way analysis of variance (ANOVA) was used to measure the significance of the difference in terms of mean bond strength between the groups, and the post hoc Tukey's honestly significant difference (HSD) test was used for pairwise comparisons. Descriptive statistics were used to report the bond strength (expressed as

the mean and the average ± standard deviation) and ARI scores (expressed as percentage and frequency). The significance of the difference in terms of ARI scores was evaluated with the Kruskal-Wallis test. Because the F statistic was significant by one-way ANOVA (F = 6.895 and p <0.001), pairwise comparisons were made between the groups using the post hoc Tukey's HSD test to determine the group or groups that caused the said difference. Statistical significance was set at p <0.05.

Results

The descriptive statistics and multiple comparisons of the SBS scores of the five groups are reported in Table 1 and Fig. 1. According to ANOVA, a statistically significant difference was found amongst the groups (F = 6.895; p <0.001). Post hoc testing revealed that G4 had the lowest mean SBS value (13.44 ± 6.37 MPa); G5, which was treated with resin infiltration, had the highest mean SBS value (22.11 ± 6.56 MPa). A bond strength of 19.09 ± 5.76 MPa was observed in the control group (Group 1). G3, in which CPP-ACPF was applied, experienced a higher bond strength than the control group (19.62 ± 5.86 MPa). The second-lowest bonding value was obtained in G2, which was the nontreated group (18.51 ± 6.31 MPa).

Kruskal-Wallis testing indicated some statistically significant differences between groups for ARI scores as well (Table 2 and Fig. 2). The difference between G1 and G4 was not significant (p = 0.038). However, the differences between G3 and G4 and between G4 and G5 were statistically significant (p <0.001 for each). When the ARI scores of all groups were examined, the difference in the distribution of the scores was statistically significant (p <0.001), because scores obtained in G4 were lower than scores in the other groups. When the other groups were compared amongst each other, the distribution of ARI scores was statistically similar (p >0.05).

Table 1. Descriptive statistics of the groups and comparison of shear bond strength values

Groups	N	Remineralization Procedure	Shear Bond Strength				ANOVA F=6,895
			Mean (MPa)	SD	Min	Max	p
Group 1	20	-	19.09[a]	5.76	9.5	29.95	p <0.001
Group 2	20	No remineralization	18.51[a]	6.31	6.62	31.34	
Group 3	20	CPP-ACPF	19.62[a]	5.86	10.75	32.11	
Group 4	20	Fluoride Varnish	13.44[b]	6.37	4.99	24.39	
Group 5	20	Resin Infiltration (ICON)	22.11[a]	6.56	9.02	29.42	

N Sample Size; SD, Standard Deviation; Min, Minimum; Max, Maximum; CPP-ACPF, Casein Phosphopeptide Amorphous Calcium Phosphate Fluoride
a, b, c, d: Different lower cases in the same column represent statistically significant differences between the groups

Table 2. Frequency distribution of the adhesive remnant index (ARI) scores of the groups

	Group 1[a]	Group 2[a]	Group 3[a]	Group 4[b]	Group 5[a]	p-value
ARI score	n(%)	n(%)	n(%)	n(%)	n(%)	
0	2 (10)	4 (20)	4 (20)	13 (65)	1 (5)	p < 0.001
1	4 (20)	4 (20)	0 (0)	4 (20)	1 (5)	
2	7 (35)	5 (25)	3 (15)	0 (0)	5 (25)	
3	2 (10)	4 (20)	3 (15)	1 (5)	4 (20)	
4	5 (25)	3 (15)	10 (50)	2 (10)	9 (45)	

n, number; Different letters show statistically significant differences.

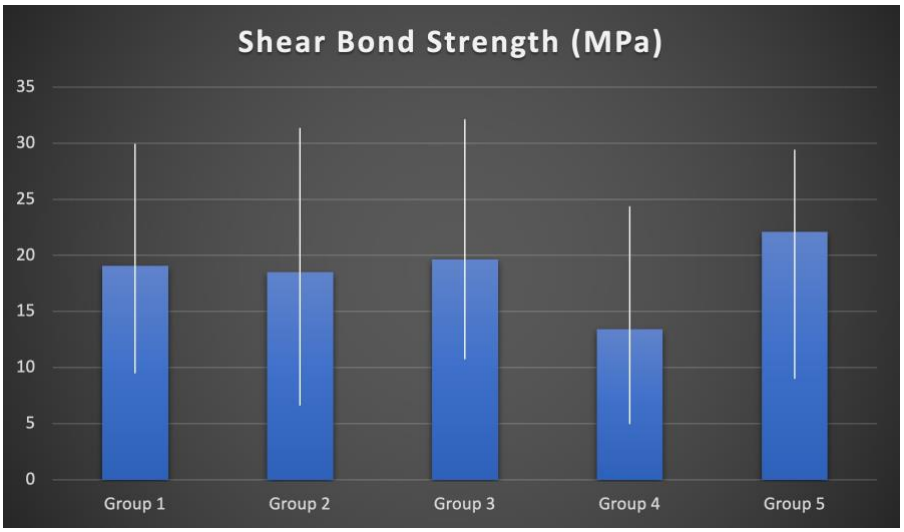

Figure 1. Statistical comparison of shear bond strength values

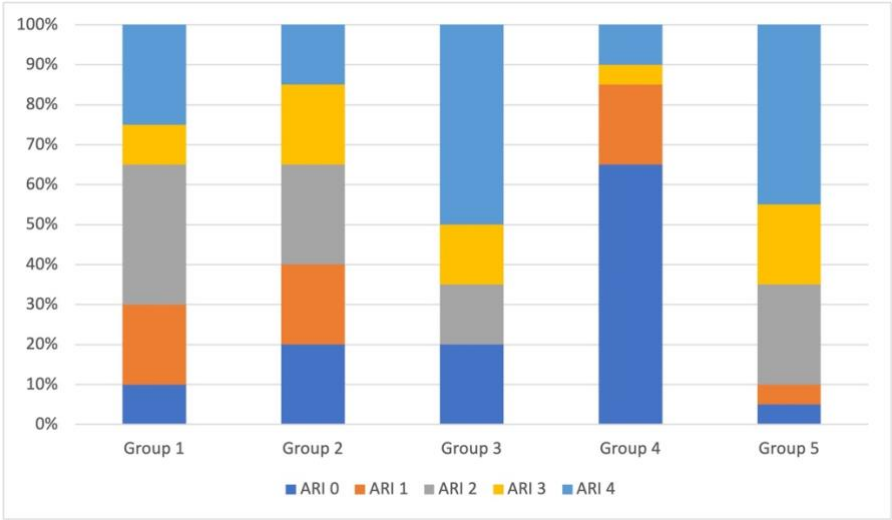

Figure 2. Distribution of adhesive remnant index scores

Discussion

To ensure the success of orthodontic treatment and patient satisfaction during active treatment with fixed orthodontic appliances, the bracket attachment values, which are directly related to the total treatment time and treatment success, must be at the desired level and WSLs must be prevented or, when present, repaired. Although studies in the literature have evaluated approaches to the treatment of WSLs that occur during orthodontic treatment (26, 28, 29), no study, to our knowledge, has evaluated the effects of the available techniques on SBS. Therefore, this study evaluated the bond strength and residual adhesive content of a control group, a demineralized enamel surface group, a CPP-ACPF, a fluoride varnish group and an ICON group.

In previous studies, the SBS values of brackets applied to intact enamel were usually higher than those of the demineralized enamel groups (28, 30, 31). In this study, higher SBS values were obtained in the intact enamel group than in the demineralized enamel group, in accordance with previous studies, but the difference was not statistically significant. However, Gulec and Goymen also found no statistically significant difference between the SBS values of brackets placed on the intact enamel surface and the demineralized enamel surface (32). One reason for these different results may be that different demineralization protocols were applied. Primary enamel lesions result from mineral losses below the superficial layer and reduce the bond strength of brackets to demineralized enamel, so enamel minerals represent an important factor for good bonding (33).

In this study, the lowest SBS values of the brackets were found in the fluoride varnish group, and these values were statistically different from those of all other groups. The lowest ARI results were also obtained with fluoride varnish. Consistent with the results of our study, Tabrizi and Cakirer found that the lowest SBS values were in the bracketed teeth treated with fluoride varnish (34). Uysal et al. found that the bracket SBS values after fluoride varnish was applied to demineralized teeth were lower than the SBS values of brackets applied to the intact enamel surface (35). In a study by Baka et al., the bracket bonding values of fluoride varnish applied to the demineralized enamel surface were close to the SBS values of the brackets applied to the intact enamel surface (31). The use of fluoride varnish replaces the calcium and phosphate ions found in the enamel prisms to transform the hydroxyapatite crystals into fluorohydroxyapatite, which is more resistant to acid attacks (36, 37). Fluorohydroxyapatite can prevent phosphoric acid etching on the enamel surface, so it may negatively affect bracket bonding because the applied bonds and resins cannot reach a sufficient depth on the enamel surface (37).

In this study, the highest bracket SBS values were found in the ICON group, and the second-highest bracket SBS value was in the CPP-ACPF group. Consistent with previous studies (28, 31, 35), our study found that the use of ICON and CPP-ACP on the demineralized enamel surface increased the SBS strength of the brackets, but there was no statistically significant difference when these two groups were compared with the intact enamel surface and demineralized enamel surface groups. Gulec and Goymen found that ICON and CPP-ACP applied to the demineralized enamel surface had brackets with SBS values lower than those on the intact enamel surface and those directly adhered to the demineralized enamel surface (32). Some reasons for this difference may include the application time of the pretreatment agents, the different waiting times in the artificial saliva solution, the use of thermal cycles and the development of bonding agents or bracket retention properties. In addition, the use of fluoride with CPP-APP in our study may have caused the differences from previous studies. In vivo and in vitro studies have shown that the use of CPP-ACP and ICON without fluoride can provide remineralization of subsurface lesions (38). Thus, these products may have increased the mineral content of tooth enamel, resulting in higher SBS scores compared with teeth that received fluoride varnish.

Daneshkazemi et al. measured the effect of CPP-ACP, fluoride varnish and resin infiltration (ICON) applications applied to intact and demineralized enamel surfaces on bracket bonding (28). Results showed that the agents applied to the demineralized enamel surface had a positive effect on bracket bonding, whereas the group in which fluoride varnish was applied had the lowest bonding strength; these results are in line with our findings. Like our study, Daneshkazemi et al. found no significant difference between ICON and CPP-ACP use in terms of bond strength (28).

Consistent with the results of this study, Ekizer et al. found that the bracket bond strength of the demineralized enamel group was lower than the strength in groups treated with ICON or CPP-ACP (26). Attin et al. obtained higher results on the intact enamel surface than on the other groups in their study (30). However, they found that ICON and fluoride varnish applied to the demineralized surface provided better bond strength than the untreated demineralized enamel surface. Baka et al. reported no significant difference between the bond strengths of ICON and CPP-ACP agents applied to intact enamel surfaces and demineralized enamel surfaces (31). Naseh et al. found no significant difference between the bracket bonding values of brackets adhered to the intact enamel surface with or without CPP-ACP and fluoride mouthwash applications (39). Differences between our research and these studies may reflect the different thermal cycle times and the different pretreatment applications used.

High ARI scores, which indicate a failure of bonding between the bracket and the composite, are more desirable because high amounts of residual adhesive remaining on the tooth surface reduce the likelihood of enamel cracks (28). In this study, the highest ARI scores were recorded in the CPP-ACP and ICON groups. The lowest ARI score was obtained in the fluoride varnish group, in which 65% of the ARI scores were 0. Cossellu et al. reported ARI scores of mostly 0 for groups with low SBS values, and these findings support the results of our study (40). In a study by Uysal et al. no enamel damage occurred in the intact enamel control group (ARI = 0). In the same study, an ARI score of 0 was achieved in 75% of the demineralized enamel group (35). Conversely, Ekizer et al. reported that ARI scores 2 and 3 were more common in demineralized enamel groups that were untreated and pretreated with ACPF gel (26). Some explanations for the different scores in different articles may be the use of different demineralization methods, differences in the amount of thermal cycling and differences in the speed of the head movement used in the universal testing devices. In laboratory environments, the creation of working conditions close to clinical conditions allows for maximum adaptation of the results obtained to the clinic. However, different factors—such as the collection time of the teeth, the storage conditions of the teeth, the method and duration of the application of the applied agents, the concentration of fluoride varnish used, the size of the slot and the base type of the bracket used—affect the results of laboratory experiments.

In this study, every effort has been made to standardise the testing procedure in order to establish a laboratory technique that is representative of the clinical setting. However, studies that perform bond strength tests in vitro may be only an indicator of the clinical performance of the tested materials, as it is difficult to precisely mimic oral conditions. Despite these limitations, such studies help identify materials for future studies.

Conclusions

Within the limitations of in vitro operating conditions, this study identified the following clinical results:

1. CPP-ACPF can be used as a prophylactic agent to prevent WSL during fixed orthodontic treatment without compromising bracket SBS.

2. ICON, however, can be used for primary lesions, because its efficacy falls between preventive and restorative treatments.

3. Before the developmental gels and resin infiltration materials enter routine clinical use, their possible side effects and their clinical performance must be evaluated.

Ethical Approval: Ethics committee approval was received for this study from Aydın Adnan Menderes University, Faculty of Dentistry Ethics Committee in accordance the World Medical Association Declaration of Helsinki, with the approval number: ADÜDHF2021 /021.

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