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In vivo effects of amorphous calcium phosphate-containing orthodontic composite on enamel demineralization around orthodontic brackets

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ABSTRACT

Background: The aim of this study was to evaluate the *in vivo* effects of an amorphous calcium phosphate-containing orthodontic composite in reducing enamel demineralization around orthodontic brackets, and to compare it with the control.
Methods: Fourteen orthodontic patients were divided into two equal groups. They received brackets fitted to all first premolars, bonded with either Aegis Ortho® (The Bosworth Co.), an ACP-containing orthodontic composite (experimental group), or Concise® (3M Dental Products), a resin-based orthodontic composite (control group). After 30 days, the teeth were extracted and longitudinally sectioned, and evaluated by superficial-microhardness analysis. The determinations were made at the bracket edge cementing limits and at occlusal and cervical points 100 and 200 μm away from the edge. In all of these positions, indentations were made at depths of 10, 20, 30, 50, 70, and 90 μm from the enamel surface. Analysis of variance (ANOVA) and Tukey *post hoc* test was used. The statistical significance level was set at $p < 0.05$.
Results: The ANOVA showed statistically significant differences for position, material, depth, and their interactions ($p < 0.001$). The multiple comparison test showed that the ACP-containing orthodontic composite was significantly more efficient than the control composite, reducing enamel demineralization in almost all evaluations ($p < 0.001$).
Conclusions: Present results indicated that ACP-containing orthodontic composite for bonding orthodontic brackets successfully inhibited demineralization *in vivo*. This effect was localized to the area around the brackets and was statistically significant after 30 days.

Keywords: Demineralization, amorphous calcium phosphate, microhardness, *in vivo*.

Abbreviations and acronyms: ACP = amorphous calcium phopsphate; ANOVA = analysis of variance; CPP-ACP = casein phosphopeptide-amorphous calcium phosphate.

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INTRODUCTION

Despite advances in orthodontic materials and techniques in recent years, the development of decay around brackets during orthodontic treatment continues to be a problem.[1] Fixed orthodontic appliances make it difficult for young patients to maintain adequate oral hygiene during orthodontic treatment.[2] Patients with fixed orthodontic appliances have an elevated risk of caries, and enamel lesions can occur within a month, irrespective of mechanical plaque control and whether fluoridated dentifrice is used.[3–6] Caries lesions around orthodontic brackets can be reduced[5,7] or even completely inhibited when a fluoride dentifrice is used with a mouthrinse.[3,5] However, the use of a mouthrinse completely depends on patient compliance, which is frequently low.[7]

Several methods have been used to prevent or reduce enamel demineralization during orthodontic treatment, including fluoride application in various forms, enamel sealants, rigorous oral hygiene regimens, using glass ionomer cement for bonding brackets and modified appliance designs.[8–11]

Demineralization takes place when specific bacteria are retained for a long time on the enamel surface.[12]

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The bacteria metabolize fermentable carbohydrates and produce organic acids. These acids dissolve the calcium phosphate mineral of the enamel and dentine, resulting in demineralization.[13] Demineralization is first observed clinically as white spot lesions. The demineralized area beneath the dental plaque and the body of the enamel lesion can lose as much as 50% of the original mineral content.[14]

In a recent study, Schumacher *et al.*[15] developed bioactive based restorative materials that may stimulate the repair of tooth structure through the release of cavity-fighting components, including calcium and phosphate. They contain amorphous calcium phosphate (ACP) as bioactive filler encapsulated in a polymer binder.[16–18] Calcium and phosphate ions released from ACP composites, especially in response to changes in the oral environment caused by bacterial plaque or acidic foods, can be deposited into the tooth structures as an apatite mineral, which is similar to the hydroxyapatite found naturally in teeth.[19]

ACP has the properties of both a preventive and restorative material that justify its use in dental cements, sealants, composites[15–19] and, more recently, orthodontic adhesives.[9,20–22] ACP-filled composite resins have been shown to recover 71% of the lost mineral content of decalcified teeth.[16] One ACP-containing adhesive, Aegis-Ortho® (The Bosworth Co., Skokie, IL, USA), has been marketed for use as a light-cured orthodontic adhesive with similar properties to previously used resins.

In vitro studies have demonstrated the remineralization potential,[12] or shear bond strength of brackets[20,21] or lingual retainer composites[22] of ACP-containing materials, but no *in vivo* studies have investigated the efficiency of ACP-containing orthodontic material on enamel demineralization around orthodontic brackets.

Therefore, the aim of this study was to evaluate the *in vivo* effects of an ACP-containing orthodontic composite in reducing enamel demineralization around orthodontic brackets and compare it with the conventional orthodontic composite. For the purposes of this study, the null hypothesis assumed that ACP-containing orthodontic composite can significantly reduce the overall amount of demineralization around orthodontic brackets, in the mouth.

MATERIALS AND METHODS

This study was approved by the Ethical Committee on Research of the Gülhane Military Medical Academy, Ankara, Turkey. Fourteen orthodontic patients, aged 12–17 years, who were scheduled to have four first premolars extracted for orthodontic reasons were invited to participate and signed a consent form. This study was organized as a parallel group design with one group receiving the experimental material and the other receiving the control. A power analysis established by G*Power Ver. 3.0.10 (Franz Faul, Universität Kiel, Germany) software, based on 1:1 ratio between groups, sample size of 14 patients would give more than 80% power to detect significant differences with 0.40 effect size and at $\alpha = 0.05$ significance level. The total sample was divided into two groups of 7 patients each. Block randomization to obtain equal numbers in each group was used. For group standardization, before starting the procedure, all patients' teeth were evaluated clinically and radiographically to determine the baseline carries risk. Six participants (43%) were male and 8 (57%) were female. The mean age was 14.34 ± 1.91 years.

Salivary flow rate and buffer capacity were determined in order to meet the inclusion criteria of absence of active carious lesions, normal salivary flow rate (greater than 1.0 mL/min) and buffer capacity (final pH between 6.0 and 7.0). All patients received a full mouth cleaning to remove plaque in preparation for bonding. There were no visible signs of caries, fluorosis, or developmental defects in the teeth used. For evaluating the baseline demineralization values of all selected teeth, a portable battery powered laser fluorescence device, DIAGNOdent Pen (KaVo, Germany) was used. The two group scores for demineralization were low and thus demonstrated their equivalency prior to the experimental condition. Orthodontic brackets were bonded with either of the bonding materials: Aegis Ortho® (The Bosworth Co., Skokie, IL, USA), an ACP-containing orthodontic composite, or Concise® (3M Dental Products, St Paul, MN, USA), a resin-based composite (control group). The manufacturers' recommendations were followed, including conventional acid etching before bonding with Aegis Ortho® and Concise®. Excessive composite around the bracket and between bracket base and tooth was removed with a clinical probe at bonding.

Stainless steel orthodontic premolar brackets (Dyna-Lok series, 100-gauge mesh, 3M Unitek, Monrovia, CA, USA) were bonded by a standard protocol. A light-emitting diode light unit (Elipar Freelight 2, 3M-ESPE, St Paul, MN, USA) was used to cure the adhesives for 20 seconds.

Twenty-eight brackets were cemented for each group (14 maxillary and 14 mandibular first premolars in both groups). No adverse advents or side effects in each intervention group were determined. After 30 days, the brackets were removed. The teeth were extracted on the same day and stored in a refrigerator in flasks containing gauze dampened with 2% formaldehyde, pH 7.0, until the analysis. Demineralization in enamel around the brackets was evaluated by cross-sectional microhardness method according to the literature.[1,6,23] During the experimental period and three weeks before it commenced, the subjects brushed their teeth with a

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non-fluoridated dentifrice, but they drank fluoridated water. They received no instructions regarding oral hygiene, kept their usual habits, and were instructed not to use any antibacterial substance.

Cross-sectional microhardness analysis

One operator, who was blind from the group allocation, carried out the microhardness analysis (SO). The roots were removed 2 mm apical to the cemento-enamel junction, and the crowns were hemi-sectioned vertically into mesial and distal halves with a 15 HC (large) wafering blade on an Isomet low-speed saw (Buehler, Lake Bluff, IL, USA) directly through the slot of the bracket, leaving a gingival and an incisal portion. The teeth were embedded in self-curing EpoKwick epoxy resin (Buehler, Lake Buff, IL, USA), leaving the cut face exposed. The half crown sections were polished with three grades of abrasive paper discs (320, 600 and 1200 grit). Final polishing was undertaken with a 1 μm diamond spray and a polishing cloth disc (Buehler). A Shimadzu (Kyoto, Japan) HMV-700 microhardness tester under a 2 N load for 15 seconds was used for the microhardness analysis.

Forty-eight indentations were made in each half crown, in eight positions, according to the definitions of Pascotto *et al.* (Fig 1).[6] On the buccal surface, indentations were made under the bracket. In the occlusal and cervical regions, indentations were made at the edge (0) of the bracket and at 100 and 200 μm away from it. Indentations were also made in the middle third of the lingual surface of each half crown, as another control. In all these positions, six indentations were made at 10, 20, 30, 50, 70, and 90 μm from the external surface of the enamel. The values of microhardness numbers found in the two half crowns were averaged. Cross-sectional microhardness was used to evaluate demineralization/caries because of the strong correlation (r = 0.91) found between enamel microhardness scores and the percentage of mineral loss in caries lesions.[24]

Statistical analysis

Data analysis was performed by using Statistical Package for Social Sciences (SPSS, Version 13.0, SPSS Inc., Chicago, IL, USA) and Excel 2000 (Microsoft Corporation, Redmond, WA, USA). Analysis of variance (ANOVA) was used to evaluate the effect of materials (Aegis Ortho® and Concise®), depths from the enamel surface (10, 20, 30, 50, 70 and 90 μm), positions (under the bracket, and on the buccal surface in occlusal and cervical regions at 0, 100 and 200 μm from the brackets and in the lingual surface), and their interactions. For multiple comparisons, the Tukey Honestly Significant Difference (HSD) test was used. The statistically significance level was set at $p < 0.05$ level.

RESULTS

The ANOVA showed statistically significant difference for the factors material, position, and depth ($p < 0.001$). The interactions (position/material, position/depth, material/depth and position/material/depth) were also statistically significant ($p < 0.001$) (Table 1).

Descriptive statistics and multiple comparisons of microhardness for materials at different depths from the enamel surface are presented in Table 2. The interaction between depth and material showed significant differences between the tested materials at the distances of 10 and 20 μm from the enamel surface. Less demineralization was found in enamel around the brackets bonded with the ACP-containing orthodontic composite in comparison with the control composite.

Descriptive statistics and the *post hoc* statistical comparisons of microhardness for materials at different positions, under and occlusal and cervical to the brackets on labial and lingual (control) surfaces are shown in Table 3. The interaction position/material showed a statistically significant difference between the materials in the cervical (0 μm and 100 μm) and occlusal (0 μm) region of the bracket ($p < 0.001$). The greatest mineral loss (lowest hardness) was observed in the cervical region (0 μm) for the control group.

The Tukey *post hoc* test applied to the triple interaction (material/position/depth) and the results are presented in Table 4. These results showed statistically significant differences at all positions evaluated on the buccal surface, but only at 10 μm from the surface of the enamel. There was no significant difference between the materials in the hardness observed at the lingual surface of the untreated teeth.

Table 1. ANOVA results and interactions for the factors material, position and depth

Source	Degrees of freedom	Sum of squares	Average square error	F	*p*
Position	7	11795.00	495.546	21.300	<0.001
Material	1	33168.00	9755.454	56.800	<0.001
Depth	5	17683.00	1456.765	36.404	<0.001
Position/material	7	2839.00	495.546	6.240	<0.001
Position/depth	35	13705.50	167.76	43.690	<0.001
Material/depth	5	25555.00	1456.765	22.264	<0.001
Position/material/depth	35	963.00	167.76	53.693	<0.001

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Table 2. Descriptive statistics and multiple comparisons of microhardness for materials at different depths from enamel surface

Interaction of depth/material	ACP-containing orthodontic composite (Aegis Ortho®)		Resin based orthodontic composite (Concise®)		Error of degree of freedom	Multiple comparisons
	Mean of different positions	SD	Mean of different positions	SD		
10 μm	280.270	26.900	178.130	29.340	0.224	p <0.001
20 μm	294.710	20.180	199.450	24.660		p <0.001
30 μm	307.310	17.690	305.230	18.720		NS
50 μm	340.730	14.010	346.870	13.540		NS
70 μm	365.820	11.210	364.610	10.960		NS
90 μm	372.920	7.100	370.430	7.560		NS

SD = standard deviation; NS = not significant.

Table 3. Descriptive statistics and *post hoc* statistical comparisons of microhardness for materials at different positions, under and occlusal and cervical to the brackets on labial and lingual (control) surfaces

Interaction of place/material	ACP-containing orthodontic composite (Aegis Ortho®)		Resin based orthodontic composite (Concise®)		Error of degree of freedom	Multiple Comparisons
	Mean of different depths	SD	Mean of different depths	SD		
Occlusal/200 μm	334.890	14.760	326.980	15.090	0.317	NS
Occlusal/100 μm	329.870	18.270	319.580	20.200		NS
Occlusal/0 μm	308.390	19.220	283.630	25.220		p <0.001
Under bracket	343.730	15.210	339.310	16.480		NS
Cervical/0 μm	303.280	18.740	279.980	27.180		p <0.001
Cervical/100 μm	323.480	20.230	287.910	24.960		p <0.001
Cervical/200 μm	333.090	17.920	324.390	16.030		NS
Lingual	331.650	16.870	329.760	17.100		NS

SD = standard deviation; NS = not significant.

Table 4. Descriptive statistics and multiple comparisons of microhardness for materials and positions at depth of 10 μm

Interaction of material/place/depth	ACP-containing orthodontic composite (Aegis Ortho®)		Resin based orthodontic composite (Concise®)		Error of degree of freedom	Multiple comparisons
	Mean	SD	Mean	SD		
Aegis Ortho/Concise/Occlusal 200 μm/10 μm	277.980	32.340	233.810	45.980	0.388	p <0.001
Aegis Ortho/Concise/Occlusal 100 μm/10 μm	255.180	29.540	213.320	49.170		p <0.001
Aegis Ortho/Concise/Occlusal 0 μm/10 μm	206.610	33.090	164.940	48.900		p <0.001
Aegis Ortho/Concise/Under/10 μm	323.730	27.990	271.380	25.090		p <0.001
Aegis Ortho/Concise/Cervical 0 μm/10 μm	202.750	45.130	134.980	27.270		p <0.001
Aegis Ortho/Concise/Cervical 100 μm/10 μm	238.490	39.280	151.010	33.870		p <0.001
Aegis Ortho/Concise/Cervical 200 μm/10 μm	258.220	33.650	247.260	38.120		p <0.001
Aegis Ortho/Concise/Lingual/10 μm	296.330	23.870	295.920	27.000		NS

SD = standard deviation; NS = not significant.

Thus, the null hypothesis that the ACP-containing orthodontic composite used for bonding brackets can significantly reduce the overall amount of demineralization around orthodontic brackets could not be rejected.

DISCUSSION

The role of bioactive material, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) in decreasing the incidence of dental caries has been investigated in many studies.[25,26] CPP-ACP involves the incorporation of the nano-complexes into dental plaque and onto the tooth surface, thereby acting as a calcium and phosphate reservoir. Studies have shown that CPP-ACP incorporated into dental plaque can significantly increase the levels of plaque calcium and phosphate ions.[25,26] This mechanism is ideal for the prevention of enamel demineralization as there appears to be an inverse association between plaque calcium and phosphate levels and

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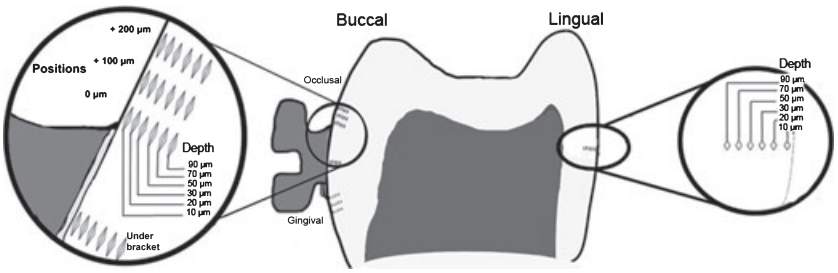


Fig 1. Diagrammatic representation of positions and depth of indentations.

measured caries experience.[25] CPP-ACP has been incorporated into various products (sugar-free chewing gums, mints, topical gels and experimentally-tested sports drinks and glass ionomer cements) in order to exert a topical effect.[27]

An ACP-containing material has been marketed for use as a light-cured orthodontic bracket bonding composite and some *in vitro* studies have been carried out to evaluate the physical[20–22] and demineralization inhibiting[12] properties of this material. This composite can be maintained under the brackets for a considerable time, offering a promising antagonist to demineralization, and can promote the prevention of future white spots throughout orthodontic treatment.[17]

The present *in vivo* study evaluated the effect of orthodontic bonding materials on demineralization in enamel around brackets. Mineral loss was assessed *in vitro* by cross-sectional microhardness, a recognized analytical method. Previously, the cariostatic effect of fluoride-releasing materials were investigated by using a split-mouth design.[28,29] However, in the present study, the subjects were randomly divided into two equal groups, and each subject received only one tested material because the baseline clinical, radiological, salivary and laser fluorescence examinations showed that the patients were equal in regards to caries risk or demineralization activity. As suggested by Pascotto *et al*.,[6] the current experimental design was chosen instead of the split-mouth technique to avoid the carry-across effect due to calcium and phosphate release by the ACP-containing composite on enamel around the brackets bonded with this composite resin.

The present model had several advantages:[23] the development of the caries lesions was studied in vital teeth; minimal patient cooperation was required; no special diet was required; and, because the protected enamel surface allowed the accumulation of thick plaque, no other site was at risk of caries with this procedure. The only disadvantage was the limited study period of 30 days because of ethical considerations, as with most other caries models. White spot lesions have the potential to develop within four weeks from the commencement of orthodontic treatment.[3,4] A 30-day experimental period was used because measurable demineralization can be observed around orthodontic appliances one month after bonding by using the microhardness test method.[3,4] The hardness values of enamel under the two internal controls (under the bracket and at the middle third lingual surface of the crowns) bonded with the two materials used to evaluate the effect of the acid etching and the individual enamel hardness.[6] Present findings showed that hardness values were statistically similar, showing that demineralization was due to caries and not the effect of acid etching.

Adapted from the method of Pascotto *et al*.[6], for an extensive and controlled evaluation, the indentations were made at 10, 20, 30, 50, 70, and 90 µm from the external surface of the enamel to observe mineral changes at the outermost part of the enamel.

Table 2 shows the development of a narrow caries lesion around brackets, with significant differences ($p < 0.001$) between two investigated composites of up to 30 µm depth from the enamel surface. Significant differences were found between the two tested composites at a depth of 10 and 20 µm from the enamel surface. The depth of the lesions was similar to previous studies,[3,6] but not in accordance with the reports of de Moura *et al*.[23] that showed lesions up to a depth of 70 µm from the enamel surface. This could be attributed to the experimental model used which allowed more plaque accumulation and impaired its removal by toothbrushing. The effect of various protective materials in reducing enamel demineralization under present conditions is supported by many *in vitro* and *in vivo* evaluations.[1,3,6] However, the present *in vivo,* short-term follow-up was the first study that showed the preventive effects of ACP containing composite, against demineralization.

In the present study, similar to previous findings,[24,28] higher mineral loss was observed at the cervical region (100 µm away from the bracket) than the occlusal. At the 0 µm level, the mean microhardness value was similar for both occlusal and cervical regions. Pascotto *et al*.[6] observed reduced enamel hardness in the cervical region of the bracket compared with that in the occlusal area. The explanation for the observation *in vivo* is the greater dental plaque accumulation and the patient's difficulty in cleaning this area.[6] *In vitro*, the explanation would be the lower mineralization and the higher carbonate on the cervical surface than in the occlusal region.[6]

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Statistically significant differences between the materials at ($p < 0.001$) level were observed in the cervical area at 0 μm and 100 μm level, but not in the occlusal region at 100 μm level. The lower hardness result at the cervical region was previously reported by Pascotto *et al.*[6] However de Moura *et al.*[23] determined that the material tested for effect on demineralization significantly increased enamel hardness in both the occlusal and the cervical regions of the bracket base. Similar to the CPP-ACP containing materials, the effect of ACP-containing orthodontic composite occurs on the tooth surface where the patient has difficulty in cleaning dental plaque by brushing. This effect can be attributed to the calcium and phosphate-releasing ability of this orthodontic composite when submitted to cariogenic challenges.

Multiple comparisons of microhardness for materials and positions at a depth of 10 μm showed that at 10 μm from the surface, the only position with no significant difference between the materials was the one on the untreated lingual surface. However, the difference in enamel hardness under the brackets bonded with Aegis Ortho® or Concise® might be attributed to the acid etching during the bonding with the resin. This effect was first described by O'Reilly and Featherstone,[3] and then by Pascotto *et al.*[6] who found mineral loss of 3% to 8% directly under the brackets retained with composite resin.

The present microhardness results show that teeth bonded with ACP-containing orthodontic composite have significantly less enamel mineral loss when compared with teeth bonded with conventional composite resin in a group of orthodontic adolescent patients. This suggests that, at least in the short term, teeth bonded with ACP-containing material are significantly more resistant to demineralization because of dental caries than those bonded with composite, even in patients known for their high caries risk. Often patients have brackets on all teeth, not just four first premolar teeth, with wires and elastics compounding the plaque build-up, so that the difference in the effect of the two composite materials would probably be even more apparent.[1]

CONCLUSIONS

With an *in vivo* tooth bracket model, it can be concluded that the use of an ACP-containing orthodontic composite significantly reduces enamel mineral loss due to dental caries around orthodontic brackets in patients' mouths compared with conventional orthodontic composites over a 30-day period.

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