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Amorphous calcium phosphate-containing orthodontic composites. Do they prevent demineralisation around orthodontic brackets?

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Background: A preliminary study using laser fluorescence suggested that amorphous phosphate-containing orthodontic composites may prevent demineralisation around bonded orthodontic brackets.
Objective: To compare the microhardness of the enamel around brackets bonded with an amorphous calcium phosphate-containing orthodontic composite (ACP-containing) with the microhardness of the enamel around brackets bonded with a conventional composite resin.
Methods: Forty extracted upper premolars were used. Orthodontic brackets were bonded to the teeth with either an ACP-containing composite resin (N = 20) or a conventional composite resin (N = 20). The latter were used as the control. The crowns of all teeth were painted with an acid resistant varnish, leaving a 2 mm ring of exposed enamel around the brackets. The teeth were then subjected to a daily cycle of demineralisation for 6 hours and remineralisation for 18 hours for 21 days. Each tooth was sectioned and the microhardness of the enamel determined 25, 50, 75, 100 and 150 μm from the surface.
Results: The enamel was significantly harder 25 μm ($p = 0.000$) and 50 μm ($p = 0.001$) from the enamel surface in the teeth with brackets bonded with the ACP-containing composite resin as compared with the control teeth.
Conclusion: ACP-containing orthodontic composite resins may reduce the enamel decalcification found in patients with poor oral hygiene.
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Introduction

Poor oral hygiene following placement of a bonded orthodontic appliance can result in unsightly white spot lesions on the labial surfaces of the anterior teeth.[1,2] Although a white spot lesion is the first visible sign of enamel softening, measurable demineralisation can occur around orthodontic brackets as early as one month after an appliance has been placed.[3–6] Overall management of white spot lesions involves methods of both preventing demineralisation and encouraging remineralisation of existing lesions.[7] The former includes good oral hygiene and the latter may include the application of materials, such as casein phosphopeptide – amorphous calcium phosphate nano-complexes (CPP-ACP) that enhance remineralisation of enamel.[8] A recent development has been the addition of amorphous calcium phosphate (ACP) to orthodontic composites with the intention of enhancing enamel remineralisation around bonded brackets. This area is particularly prone to demineralisation because plaque readily accumulates on orthodontic composites.

In a preliminary study we used a laser fluorescence device to compare 'enamel demineralisation' around orthodontic brackets bonded with either an ACP-containing composite or a resin-modified glass ionomer cement.[9] We found that ACP-containing orthodontic composites provided the highest reductions in enamel demineralisation when compared with a resin-modified glass ionomer cement and the control. However, Diniz et al. recently reported that laser fluorescence devices for detecting in-vitro demineralisation are unreliable.[10] Thus, we decided to determine the extent of demineralisation around orthodontic brackets bonded with either an ACP-containing composite resin or a conventional composite resin by measuring enamel microhardness: one of the traditional methods of determining early demineralisation.[4–6,10] The method, which has been widely used in caries research, correlates demineralisation with microhardness.[4]

The aim of this in-vitro study was to compare the microhardness of the enamel around brackets bonded with an amorphous calcium phosphate-containing orthodontic composite (ACP-containing) with the enamel around brackets bonded with a conventional composite resin after the teeth had been exposed to acid attack.

Materials and methods

Preparation of the teeth

Forty caries-free human upper premolars, extracted for orthodontic reasons, were used in this study. Before use the teeth were stored in 0.1 per cent thymol for periods no longer than one month. Teeth with hypoplastic areas, cracks and/or gross irregularities of enamel structure or treated with chemical agents such as alcohol, formalin or hydrogen peroxide were excluded from the study. Soft tissue remnants and calculus were removed from the teeth and the crowns cleaned with fluoride-free pumice and a rubber cup. The teeth were then stored in Streck Tissue Fixative (Streck Laboratories, Inc., Omaha, NE, USA) for two weeks. This solution has an antimicrobial action and does not compromise histological examination of the enamel following artificial demineralisation.[11] The teeth were randomly distributed into the control (Group 1) and the experimental group (Group 2) equally. The buccal surface of each premolar was etched with 37 per cent orthophosphoric acid gel (3M Dental Products, St. Paul, MN, USA) for 15 seconds, rinsed with water for 15 seconds and dried with oil-free air for 10 seconds until the enamel appeared frosty-white.

In Group 1, Transbond XT primer (3M Unitek, Monrovia, CA, USA) was applied to the etched surface in a thin film and not cured. Transbond XT composite paste was applied to the base of the stainless steel bracket (Dyna-Lok series, 100-gauge mesh, 3M Unitek, USA). Each bracket was positioned at the maximum contour mesio-distally in the middle one third of the buccal surface occluso-gingivally and parallel to the long axis of the tooth, and pressed firmly into place. The excess composite was removed with a scaler.

In Group 2, a thin layer of ACP-containing orthodontic composite (Aegis Ortho, Harry J Bosworth Co., IL, USA) was applied to the etched enamel and the base of the bracket. The bracket was pressed onto the buccal surface of the tooth in an identical position to that used in Group 1. Following the manufacturer's recommendations, excess composite was not removed.

A light-emitting diode curing unit (Elipar Freelight 2, 3M-ESPE, St. Paul, MN, USA) was applied to the mesial and distal edges of the brackets in both groups for 10 seconds per side (Total time: 20 seconds). Following curing, the crowns of the teeth were painted with an acid-resistant varnish, leaving a 2 mm ring of exposed enamel around the brackets. The specimens were stored in 100 per cent humidity for 12 hours at 37 °C.

Demineralisation procedure

The baseline mineralisation of the enamel around each bracket was measured with a portable battery-powered laser fluorescence device (Diagnodent Pen, KaVo, Germany). The scores in both groups were under 13, indicating that the enamel was not demineralised and all teeth had the same risk of caries (Figure 1).

The demineralisation procedure was adapted from the method described by Hu and Featherstone.[12] The daily procedure of pH cycling included a demineralisation period of 6 hours (0900–1500 hours) and a remineralisation period of 18 hours (1500–0900 hours). The crown of each tooth was immersed in 60 mL of demineralisation solution containing 2.0 mmol/L calcium, 2.0 mmol/L phosphates, and 75

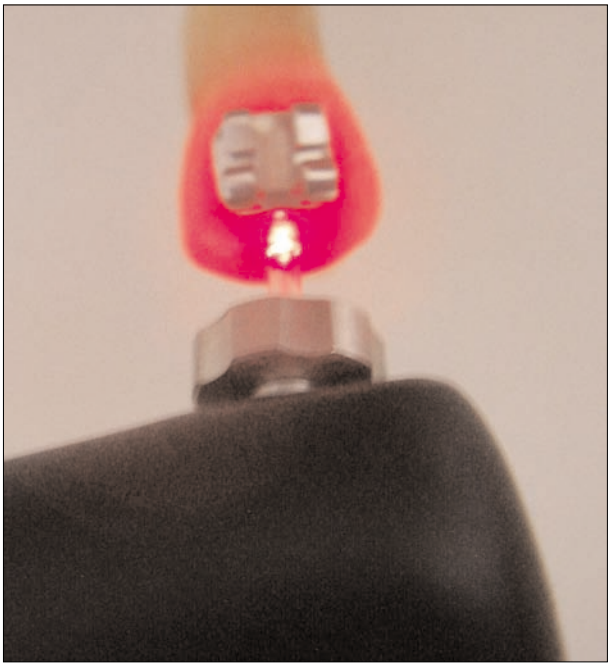

Figure 1. Measurement of the demineralisation on the occlusal side of the bracket by Diagnodent Pen.

mmol/L acetate at pH 4.3 for 6 hours at 37 °C. Specimens were then removed from the demineralisation solution, rinsed with deionised water and immersed in 40 mL of the remineralisation solution at 37 °C overnight (18 hours), to simulate the remineralising stage of the caries process. The remineralisation solution consisted of 1.5 mmol/L calcium, 0.9 mmol/L phosphates, 150 mmol/L potassium chloride, and 20 mmol/L cacodylate buffers at pH 7.0. This cycling procedure was repeated daily for 21 days. On day 21, the presence of demineralised enamel was confirmed with the laser fluorescent device and, visually, the enamel appeared frosty-white when the teeth were dried. The teeth were removed from the solution and the brackets removed.

Microhardness analysis

The roots of the teeth were removed with a water-cooled diamond disk. The crowns were hemi-sectioned vertically into mesial and distal halves with a large 15 HC wafering blade in an Isomet low-speed saw (Buehler, Lake Bluff, IL, USA). The hemi-sections were cut into a cervical portion and an occlusal portion. Both portions were embedded in self-curing epoxy resin (Epo-Kwick, Buehler, Lake Bluff, IL, USA), leaving the cut face exposed. The half crown sections were polished with abrasive paper discs (320, 600, and 1200 grit) and polished with a 1 µm diamond spray and a cloth polishing disc (Buehler, Lake Bluff, IL, USA). A microhardness tester (HMV-700, Shimadzu, Kyoto, Japan) under a 2N load for 15 seconds was used for the microhardness analysis. In the occlusal and cervical regions, indentations were made at the edge (0 µm) of the composite and 25, 50, 75, 100, 125, and 150 µm from the external surface of the enamel.

Table I. Intra- and inter-examiner agreement for the microhardness scores.

Observation	Kappa score (K)
Intra-examiner agreement (Examiner 1)	0.83
Intra-examiner agreement (Examiner 2)	0.88
Inter-observer agreement	0.81

Cohen's Kappa:
K < 0.40 poor agreement
K = 0.41 - 0.60 moderate agreement
K = 0.61 - 0.80 substantial agreement
K > 0.80 - almost perfect agreement

Statistical analysis

Data analysis was performed by using Statistical Package for Social Sciences, (SPSS, Vers.13.0, SPSS Inc. Chicago, IL, USA) and Excel 2000 (Microsoft Corporation, Redmond, WA, USA). Descriptive statistics were calculated for both groups. The Shapiro-Wilks normality test and Levene's variance homogeneity test were applied to the microhardness data. The data were normally distributed and there was homogeneity of variances between the groups. Occlusal and cervical microhardness scores at the same depths in matching half crown specimens were compared with the paired *t*-test. Student's *t*-test was used to compare the effect of the materials (Transbond XT and Aegis Ortho) 25, 50, 75, 100, 125, and 150 µm from the enamel surface. For multiple comparisons, the analysis of variance (ANOVA) and Tukey Honestly Significant Difference (HSD) post-hoc test were used. A significance level of $p < 0.05$ was used for all tests.

To determine the intra- and inter-observer agreement, the microhardness of the enamel was measured by two investigators using the same instrument at two separate times, and Cohen's Kappa scores were determined.

Table II. Comparisons of enamel microhardness.

Depth	Composite	N	Microhardness (VHN) Mean	SD	SEM	Minimum	Maximum	p
25µm	Transbond XT	20	185.80	9.95	2.22	160	209	**0.000**
	Aegis Ortho	20	223.35	11.03	2.47	201	245	
50µm	Transbond XT	20	234.70	14.77	3.30	211	256	**0.001**
	Aegis Ortho	20	251.15	13.44	3.01	231	273	
75µm	Transbond XT	20	253.70	5.26	1.18	247	265	0.107
	Aegis Ortho	20	256.70	6.18	1.38	239	263	
100µm	Transbond XT	20	271.35	8.20	1.83	261	288	0.654
	Aegis Ortho	20	272.45	7.18	1.61	261	286	
125µm	Transbond XT	20	295.95	13.11	2.93	275	322	0.943
	Aegis Ortho	20	295.65	13.36	2.99	276	319	
150µm	Transbond XT	20	300.50	5.23	1.17	293	310	0.118
	Aegis Ortho	20	302.85	3.99	0.89	294	310	

Significant values in bold

Results

The intra- and inter-examiner Kappa scores for assessment of microhardness exceeded 0.80 (Table I).

There were no statistically significant differences between the enamel microhardnesses in the occlusal and cervical sections in each group ($p > 0.05$). The mean microhardness scores in the Transbond XT group (Group 1) increased steadily from 185.80 Vickers Hardness Number (VHN) at the 25 µm, 234.70 VHN at 50 µm, 253.70 VHN at 75 µm, 271.35 VHN at 100 µm, 295.95 VHN at 125 µm to 300.50 VHN at 150 µm (Table II). The mean microhardness scores in the Aegis Ortho group (Group 2) also increased steadily from 223.35 VHN at 25 µm to 302.85 VHN at 150 µm. Although the mean differences between the two groups fell from 37.55 VHN at 25 µm to 2.35 VHN at 150 µm, only the microhardness values at the 25 and 50 µm levels were significantly different. The enamel was significantly harder in Group 2 (Aegis Ortho) than in Group 1 (Transbond XT) 25 and 50 µm from the enamel surface. There were no significant group differences in enamel microhardness from 75 to 150 µm from the enamel surface.

The microhardness scores in both groups at the 25 and 50 µm were approximately twice as variable as the microhardness scores at the 75, 100 and 150 µm levels, but not at the 125 µm level. For example, the range in enamel microhardness 25 µm from the surface was 49 VHN in the Transbond XT group (Range: 160–209 VHN) and 44 VHN in the Aegis Ortho group (Range: 201–245 VHN). Similar variation in the ranges occurred at the 50 µm level.

Discussion

We subjected teeth with stainless steel orthodontic brackets bonded with either an ACP-containing composite resin or a conventional composite resin to cycles of demineralisation and remineralisation and measured the microhardness of the enamel surrounding the brackets. The enamel was significantly harder 25 µm and 50 µm from the enamel surface in the teeth with brackets bonded with the ACP-containing composite resin, suggesting that ACP-containing composite resins may lessen the risk of demineralisation in patients with poor oral hygiene.

In this in-vitro study we attempted to simulate the processes of acid attack that occurs beneath plaque in vivo and subsequent remineralisation by minerals in the saliva over 21 days.[12,13] Whereas Hu and Featherstone[12] cycled the teeth in their study through demineralisation and remineralisation solutions for 14 days, we extended our study to 21 days because we found no visual evidence of demineralisation after 14 days exposure to the solutions. By 21 days, however, the enamel surrounding the brackets in the control group appeared frosty-white when dried.

We assessed mineral loss from the teeth by determining the microhardness of the enamel at selected depths. This is a proven and widely-used method to determine the loss of mineral that is a feature of early carious lesions. A strong correlation (r = .91) was reported by Featherstone and coworkers between enamel microhardness scores and the percentage loss of mineral in the carious lesions.[13] While others have reported that the demineralisation of the enamel around orthodontic brackets can extend as far as 75 µm below the enamel surface, Gorton and Featherstone[4] and Pascotto et al.[5] reported that enamel demineralisation extended to only 30 µm from the enamel surface in vivo. They allowed their subjects to brush their teeth which presumably removed some/all of the plaque and micro-organisms responsible for demineralisation.

We used a portable battery-powered laser fluorescence device to determine the levels of mineralisation at the start of the study and after 21 days. Although the device confirmed that the groups had similar baseline levels of mineralisation at the start of the study and that they were demineralised after 21 days exposure to the solutions, these results should be regarded with caution as it has recently been reported that this method of determining the level of mineralisation is unreliable.[10]

Bioactive materials, such as ACP or CPP-ACP have been added to several materials used in dentistry to increase the concentrations of calcium and phosphate ions in dental plaque and replace minerals lost from the enamel during the demineralisation process.[14–17] CPP-ACP has been added to various products, such as sugar-free chewing gum, mints, topical gels, mousse, tooth paste, sports drinks and glass ionomer cements and remineralisation of already demineralised enamel lesions has been reported.[8,12,18] These innovations are ideal for the prevention of enamel demineralisation in vivo as the material is in close proximity to the enamel, and there appears to be an inverse relationship between calcium and phosphate levels in dental plaque and caries experience.[17] Sodium fluoride either alone or in combination with CPP-ACP will also prevent enamel demineralisation adjacent to orthodontic brackets.[7] The consensus is that ACP-containing materials have higher remineralising potential than the conventional composite resins and cements.[8,16]

Conclusion

ACP-containing orthodontic composite resins may reduce the enamel decalcification found in patients with poor oral hygiene.

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