Original Article

Effectiveness of varnish with CPP–ACP in prevention of caries lesions around orthodontic brackets: an OCT evaluation


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Summary

Objective: To evaluate the in vitro efficiency of applying varnish containing casein phosphopeptide (CPP) and amorphous calcium phosphate (ACP) in prevention of caries lesions around orthodontic brackets.

Materials and methods: For this purpose, brackets were bonded to the vestibular surface of bovine incisors, and eight groups were formed (n = 15) according to exposure of oral hygiene substances and enamel varnish: 1 (control) brushing only performed, 2 (control) brushing + use of mouth wash with fluoride, 3 Duraphat varnish application only (Colgate-Palmolive Ind. E Com. Ltda, São Paulo, SP, Brazil), 4 Duraphat + brushing, 5 Duraphat + brushing + mouth wash, 6 MI Varnish application (GC America, USA), 7 MI + brushing, and 8 MI + brushing + mouth wash. The experimental groups alternated between pH cycling and the procedures described and were kept in an oven at temperature of 37°C. Both brushing and immersion in solutions was performed in a time interval of 1 minute, followed by washing in deionized water three times a day for 28 days of experimentation. Afterwards, evaluation by optical coherence tomography (OCT) of the special type (Ganymede OCT/Thorlabs, Newton, USA) was performed. In each group, a scanning exam of the white spot lesion area (around the region where brackets were bonded) and depth measurement of caries lesions were performed.

Results: Groups 1 and 3 were shown to differ statistically from groups 5, 6, 7 , and 8 (p = 0.000). MI Varnish was shown to be more effective in diminishing caries lesion depth, compared with Duraphat, irrespective of being associated with brushing and mouthwash, or not.

Limitations: The major limitation of this study is that it is a study in which demineralization was obtained with the use of chemical products, and did not occur due to the presence of Streptococcus mutans and its acid byproducts.

Conclusion: Application CPP–ACP-containing varnish irrespective of being associated with brushing and mouthwash, or not, reduced depth of caries lesions around orthodontic brackets.
Introduction

White spot lesions are a problem commonly found in patients who use orthodontic devices. While it takes at least 6 months for caries to develop in a patient not submitted to orthodontic treatment, it takes around 1 month (1,2) to develop in an orthodontic patient, because of the difficulty these patients have with performing oral hygiene (3). This datum shows the rapid progression of this disease and the need for continual follow-up of these patients (1).

As has been mentioned previously, enamel demineralization (white spot) develops around orthodontic brackets, ligatures, and bands because these appliances make it difficult to perform cleaning, and potentiate bacterial biofilm accumulation on tooth surfaces (1,2,4).

This occurs due to an imbalance between the demineralization and remineralization processes performed by saliva (5–7). These reactions occur in the oral cavity all the time, and with the purpose of helping to balance them fluoride is used, which strengthens the enamel structure and makes it less susceptible to demineralization (6).

Although there are many methods for the effective control of this initial stage of caries disease, prevention of white spot lesions is a great challenge to orthodontists (8).

The use of fluoride-releasing materials (adhesives, elastics) or even topical application of this ion (varnishes, sealants, gels, dentifrices and mouth washes) are important adjuvants in clinical practice, suggested as a method for the prevention of demineralization, particularly when the patient’s level of cooperation is not ideal (2,4,8).

Over the last few years, agents have been developed which, under cariogenic conditions, release substances that help in the remineralization of enamel and dentin. There are new products that have casein phosphopeptide (CPP) and amorphous calcium phosphate (ACP) in their composition, a protein derived from milk, which acts on reducing demineralization and promotes remineralization of caries lesions when they are incorporated into the tooth surface of dental biofilm (9).

In this context, a question arises: would these materials be capable of reducing demineralization in patients using fixed orthodontic appliances? Based on this premise, the authors’ proposal in this study was to evaluate the efficacy of varnish with CPP–ACP in the prevention of white spot lesions around orthodontic devices, when submitted to pH cycling and to test the hypothesis that the varnish with CPP–ACP is more effective than the conventional varnishes for preventing white spots around the orthodontic accessories.

Materials and methods

One hundred and twenty bovine incisors were used. They were stored in a 10% formaldehyde solution for 15 days, under refrigeration at 5°C. After removing the remaining periodontal ligament, test specimens were fabricated by inclining the teeth in polyvinylchloride forms (Amanco, São Paulo, Brazil), to enable a larger area of exposure. They were fixed with self-polymerizing acrylic resin (Jet, Artigos Odontológicos Clássico Ltda, São Paulo, SP, Brazil). After resin polymerization, each test specimen containing 15 bovine teeth was abraded with silicon carbide abrasive paper (grain 600M, 3M, Rio de Janeiro, RJ, Brazil), under water lubrication, so that the vestibular surface of interest was exposed by removal of the excess resin and flattening of the enamel. With a view to maintaining standardization, the abrasion process was performed simultaneously by applying a constant force of 0.5 kg.

After fabricating the test specimens (Supplementary Figure 1), enamel prophylaxis was performed with a mixture of pumice stone and water, using a rubber cup (KG Sorensen, Rio de Janeiro, RJ, Brazil) at low speed for 15 seconds. After this, the samples were washed with deionized water for 15 seconds and then dried with a jet of oil-free air for 15 seconds.

Next, the tooth enamel surface was divided into two parts, one of which was protected with a coat of nail varnish (RISQUE, São Paulo, SP, Brazil), keeping it isolated from the experimental solutions, and the other was used for bonding 120 orthodontic brackets. Red nail varnish was used to make it easy to identify the surface to be maintained whole. For bonding the orthodontic devices, light polymerizing composite (Transbond XT, 3M Unitek, Monrovia, California, USA) was used. Before bonding, enamel was etched with 37% phosphoric acid, followed by application of adhesive (Transbond XT, 3M Unitek, Monrovia, California, USA), followed by its curing for 20 seconds. The area it was glued the bracket was insulated to prevent both the acid and the adhesive does not contaminate the area around the bracket.

After bonding, the substances to be tested were applied around the brackets. Two varnishes were used: ‘MI Varnish’ [5% sodium fluoride varnish with Recaldent (CPP–ACP), GC America, USA] and ‘Duraphat’ [5% sodium fluoride varnish, Colgate-Palmolive Ind. E Com. Ltda, São Paulo, SP, Brazil]. The application of Duraphat and MI Varnish was performed in the same manner as directed by the manufacturer, that is held after prophylaxis with pumice and water for 15 seconds, followed by drying for the same period; the varnish around the bracket was evaluated with applicator brush.

To induce the cariogenic challenge, the samples were divided into eight groups: 1: control, in which brushing only was performed; 2: control, brushing + mouth wash; 3: Duraphat, only immersion in demineralizing and remineralizing solution; 4: Duraphat + brushing; 5: Duraphat + brushing + mouth wash; 6: MI Varnish, immersion only; 7: MI Varnish + brushing; and 8: MI Varnish + brushing + mouth wash.

All the samples were submitted to brushing with fluoridated dentifrice Sorriso Dentes Brancos, 1450 ppm of fluoride (Colgate-Palmolive Ind. E Com. Ltda, São Paulo, SP, Brazil) and pH cycling. The mouth wash used was Colgate Plax Classic, 225 ppm of fluoride (Colgate-Palmolive Ind. E Com. Ltda, São Paulo, SP, Brazil). To standardize the brushing device was developed that contained soft head toothbrushes (Bitufo, Itupeva, São Paulo, Brazil) attached to the bottom of a glass box (Supplementary Figure 2). The force imposed for brushing was 1.5 N, standardized by an electrical equipment connected the glass box containing brushes (10).

This procedure was interspersed with washing the samples with water. Brushing and immersion were performed for 1 minute, followed by washing in deionized water three times a day during a period of 28 days.

pH cycling protocol

The pH cycling protocol consisted of using artificial remineralizing in neutral saliva (calcium 1.54 mmol/l; phosphate 1.54 mmol/l; acetic acid 20 mmol/l, and 0.308 g of ammonium acetate, pH adjusted to 7.0 with potassium hydroxide (VETEC, Rio de Janeiro, RJ, Brazil), and demineralizing saliva (3 mmol/l of calcium; 3 mmol/l of phosphates; 50 ml acetic acid/l; ammonium acetate and 0.308 g with pH adjusted to 4.5 with sodium hydroxide (VETEC, Rio de Janeiro, RJ, Brazil).

To induce a strong cariogenic challenge, the test specimens were stored in demineralizing saliva for 22 consecutive hours. After having been washed with deionized water, they were kept in contact with remineralizing saliva for 2 hours, in order to complete the
Evaluation by optical coherence tomography
The enamel microstructure was evaluated by means of optical coherence tomography (OCT) using a commercial system of the spectral type (Gamyned OCT/Thorlabs, Newton, USA). The system is based on the Michelson interferometer. It is connected to a pre-configured computer and the images are obtained by means of scanning. The base unit contains a light source, which in this case is a superluminescent diode whose wavelength is centered on 930 nm, with a spectral width of 100 nm. With an A-scan rate of 29 kHz, this system is able to produce 29 frames per second (fps) with 512 lines per frame and an axial resolution of 5 μm. Thus, volumetric images (3D images) in transverse cuts (2D images) were produced from a scanning exam of the white spot area located around the brackets.

Afterwards, three linear measurements were made, necessarily in different regions of each sample, corresponding to the three regions of greater depth of caries lesions identified during scanning, then the arithmetical mean of the three measurements was calculated, with this mean being representative of the depth of caries lesions for each sample.

Statistical analysis
Statistical analysis was performed with the software program SPSS 13.0 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics, which included mean (μm) and standard deviation, were calculated for all of the eight groups. Analysis of variance was applied to determine whether there were significant differences among the groups. For the post hoc test, the Tukey test was used.

Results
The results demonstrated statistically significant differences between groups 1, 3, and 4 and groups 5, 6, 7, and 8. The groups that received the application of MI Varnish showed the lowest mean depth (μm) of caries lesions, both when used alone and when associated with brushing and mouth wash (Table 1 and Figures 1–3).

Discussion
Demineralization area is a common problem after orthodontic treatment has been performed. Nowadays, there are various materials available, promising to minimize this problem (11,12).

Different forms of treatment for the prevention of these lesions have been indicated, especially for orthodontic patients, because after the conclusion of treatment, many of these lesions may remain, and this is cause for concern to patients (13). In addition to oral health promotion and education of the patient, these methods include the adoption of measures such as the application of appropriate preventive medications (14).

Many researches have suggested that topical fluoride in the form of dentifrices, gels, mouth washes, and varnishes may reduce or eliminate tooth enamel decalcification around orthodontic brackets; however, due to the wide diversity of materials on the market, it is difficult for the clinician to determine which product is the most effective (13).

Table 1. Mean values (μm), standard deviation, and corresponding statistics of linear measurements of caries lesion depths among groups evaluated

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Mean (SD)</th>
<th>Statistical differences between groups (*)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>B</td>
<td>402.82 (78.1)</td>
<td>1–2 (p = 0.334) 1–3 (p = 1.000) 1–4 (p = 0.807) 1–5 (p = 0.000) 1–6 (p = 0.000) 1–7 (p = 0.000) 1–8 (p = 0.000)</td>
</tr>
<tr>
<td>2</td>
<td>B + MW</td>
<td>340.54 (41.64)</td>
<td>2–3 (p = 0.638) 2–4 (p = 0.995) 2–5 (p = 0.149) 2–6 (p = 0.083) 2–7 (p = 0.080) 2–8 (p = 0.098)</td>
</tr>
<tr>
<td>3</td>
<td>D</td>
<td>389.79 (64.14)</td>
<td>3–4 (p = 0.969) 3–5 (p = 0.001) 3–6 (p = 0.000) 3–7 (p = 0.000) 3–8 (p = 0.000)</td>
</tr>
<tr>
<td>4</td>
<td>D + B</td>
<td>361.24 (134.09)</td>
<td>4–5 (p = 0.020) 4–6 (p = 0.010) 4–7 (p = 0.009) 4–8 (p = 0.012)</td>
</tr>
<tr>
<td>5</td>
<td>D + B + MW</td>
<td>266.98 (61.43)</td>
<td>5–6 (p = 1.000) 5–7 (p = 1.000) 5–8 (p = 1.000)</td>
</tr>
<tr>
<td>6</td>
<td>MI</td>
<td>260.22 (90.10)</td>
<td>6–7 (p = 1.000) 6–8 (p = 1.000)</td>
</tr>
<tr>
<td>7</td>
<td>MI + B</td>
<td>259.79 (51.05)</td>
<td>6–8 (p = 1.000)</td>
</tr>
<tr>
<td>8</td>
<td>MI + B + MW</td>
<td>261.98 (41.84)</td>
<td></td>
</tr>
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</table>

B = brushing; B + MW = brushing associated with mouth wash; D = Duraphat; D + B = Duraphat + brushing; D + B + MW = Duraphat + brushing + mouth wash; MI = MI Varnish; MI + B = MI Varnish + brushing; MI + B + MW = MI Varnish + brushing + mouth wash; SD = standard deviation.

*Statistically significant differences (p < 0.05).
In order to simulate a situation closer to that found clinically, we performed a pH cycling protocol (19). This method proves to be simple and effective in simulating a highly cariogenic situation, a situation common in patients with poor oral hygiene.

According to Chadwick et al. (13), the use of topical fluoride combined with fluoridated dentifrices appears to reduce the enamel demineralization in patients undergoing orthodontic treatment.

In this research, the analysis of samples revealed that the surfaces covered with Duraphat presented deeper carious lesions compared with those on which MI varnish was applied (Table 1). It was observed that Duraphat varnish was not effective when combined with brushing only. Its potential to reduce white spot lesions was only perceived when it was associated with brushing and fluoridated mouth wash. This dependence was not noted with MI Varnish, which showed excellent performance whether associated with brushing and mouth wash or not. These results may be justified by the adherence of the materials to the enamel surface. The adherence of Duraphat to enamel may have been better, so that when it was associated with brushing and mouth washes, the results were improved. On the other hand, the MI Varnish may have been detached from the enamel surface with brushing, reducing its effectiveness; however, these results were not felt due to the compensation of this negative effect by the brushing and mouth washing. The mechanical effect of mouth washing could be proved in group 8 in which, although there were no statistically significant differences with group 7, in which MI Varnish and brushing were used, there was a greater depth of lesions, demonstrating debonding of sealant reaffirming this hypothesis.

In the study conducted by Du et al. (20), in which Duraphat was compared with saline solution as regards the ability to prevent carious lesion progression, with evaluations made on the first day and at the time intervals after 3 and 6 months of orthodontic bracket debonding, duraphat was shown to be more capable of preventing the progression of these lesions after the measurements at 3 and 6 months. Compared with other varnishes, such as Duraflur, Fluorniz, and others in the research developed by Santos Lde et al. (21), in spite of Duraphat not presenting the capacity to completely prevent caries formation, it demonstrated greater ability in reducing the depth of lesions, a fact that was not proved in this study.

Seeing that the two varnishes (MI and Duraphat) presented the same fluoride concentration, a probable explanation for the difference between them may be in their composition, although this was not elucidated in the tests performed. This fact is supposed because, MI Varnish has CPP–ACP as one of its components that greatly increased the anti-caries mechanism of the product and considerably diminished the depth of white spot lesions.

On the market, there are other products containing CPP–ACP, such as MI Paste Plus (GC America, Alsip, Ill, 900 ppm of fluoride, and CPP–ACP), from the same manufacturer as MI Varnish. In the study conducted by Huang et al. (22), MI Paste Plus did not have a
better performance in combating caries compared with fluoridated varnish PreviDent (Colgate Oral Pharmaceuticals, New York, USA) which does not contain the protein, and with the control group performing standard oral hygiene, nevertheless, in this study, the MI Varnish was shown to be more effective. This result can be attributed to the presentation of material in the form of varnish and not in the form of toothpaste.

Akin and Basciﬁci (15) conducted a research in post-orthodontic patients, and who developed white spot lesions were divided into four groups (control group, group that received ﬂuoridated mouthwash, group treated with topical application of CPP-ACP, and microabrasion group). The results showed that microabrasion was the best way of aesthetically treating white spot lesions; however, the topical application of CPP–ACP was very effective in reducing the size of these lesions and increasing remineralization. This result has stimulated the use of products with this component, due to its capability to diminish the degree of enamel demineralization and strengthen its structure in the remineralization process.

No statistical differences were found between the groups which had just brushing mouthwash and the group which had only the application of MI Varnish. This result demonstrates the effectiveness of this material in preventing the progression of carious lesions without the need to use other methods associated with hygiene. Nevertheless, it is important to point out that the demineralization that occurred here was promoted by chemical agents and not by the presence of bacteria, especially Streptococcus mutans. Under clinical conditions, brushing and use of mouth washes is important because they will promote a reduction in the number of microorganisms. On the other hand, because of its mechanical action, brushing would promote sealant deterioration, thus reducing the efﬁcacy of the sealant. The fact that there was no presence of microorganisms during the process of demineralization is shown to be the major limitation of this study.

According to Ferreira et al. (23), the remineralization of white spot lesions with the use of ﬂuoridated varnish helps with the action of ﬂuoride contained in the dentiﬁres used for oral hygiene, by potentiating the protection of enamel in the remineralization process. These data are shown in this investigation.

Although the results showed that only MI Varnish used without any other oral hygiene procedure would be capable of signiﬁcantly reducing the depth of carious lesions, according to Almeida et al. (24), and Chadwick et al. (13), the beneﬁts associated with ﬂuoride and oral hygiene in the reduction of demineralization area cannot be overlooked and the success of therapy with ﬂuoride does depend on the motivation of patients as regards the quality of their oral health.

Therefore, this investigation showed that the application of adjuvant agents is a very useful tool in the elimination or reduction of dental demineralization during orthodontic treatment.

**Conclusion**

Having conducted this study, it could be concluded that the varnish with CPP–ACP was capable of reducing tooth enamel demineralization in patients who use orthodontic appliances and that the hypothesis formulated was conﬁrmed, since the varnish containing CPP–ACP was shown to be more effective than the conventional varnish used to prevent white spot lesions around orthodontic brackets.

**Funding**

Conselho Nacional de Desenvolvimento Cientíﬁco e Tecnológico (CNPq).

**References**


**Supplementary material**

Supplementary material is available at European Journal of Orthodontics online.


