Prevention of enamel demineralization during orthodontic treatment: An in vitro comparative study

Yashodhan M. Bichu, MDS¹
Nandini Kamat, MDS²
Pavan Kumar Chandra, MDS, MDSc³
Aditi Kapoor, MDS⁴
N.K.S. Aravind, MDS⁵

Aim: Enamel demineralization is considered to be the most prevalent and significant iatrogenic effect associated with fixed orthodontic treatment and can seriously jeopardize both tooth longevity and dental esthetics. This in vitro study was undertaken to compare the effectiveness of four different commercially available surface treatment medicaments for the inhibition of enamel demineralization. Methods: Seventy-five intact maxillary premolars extracted from patients undergoing orthodontic treatment were divided into five equal groups and were subjected to one of the following protocols: no treatment (control group) or treatment with one of the following four medicaments: fluoride varnish (Fluor Protector [FP]), casein phosphopeptide–amorphous calcium phosphate (GC Tooth Mousse [TM]), calcium sodium phosphosilicate (SHY-NM), and casein phosphopeptide–amorphous calcium phosphate with fluoride (GC Tooth Mousse Plus [TMP]). All the teeth were subjected to ten Cate demineralization solution for 96 hours and subsequently evaluated under polarized light microscopy to obtain the mean depths of enamel demineralization. One-way analysis of variance and Bonferroni comparison tests were used to obtain statistically significant differences between the five different groups at $P < .05$. Results: All four surface treatment medicaments provided statistically significant reduction in the depths of enamel demineralization as compared with the control group. FP provided the greatest protection of enamel surface in terms of reduction of lesion depth, followed by TMP, SHY-NM, and TM. Conclusions: The use of these commercially available medicaments could prove to be beneficial for patients undergoing orthodontic treatment and who are at a risk for developing enamel decalcification. ORTHODONTICS (CHIC) 2013;14:e22–e29. doi: 10.11607/ortho.870

Key words: prevention, enamel demineralization, surface treatment medicaments, orthodontic treatment

Enamel demineralization constitutes the most prevalent and significant iatrogenic effect associated with orthodontic treatment.¹ Since the translucency of enamel is directly related to its degree of mineralization, initial demineralization manifests clinically as a white spot lesion (WSL).² In the cariogenic environment adjacent to orthodontic appliances, WSLs can rapidly progress to produce frank cavitations. Although some lesions return to an
acceptable appearance after the completion of orthodontic treatment, many persist, resulting in esthetically unacceptable treatment results.\(^2\) Thus the diagnosis, prevention, and treatment of WSLs are crucial to prevent tooth decay as well as compromised dental esthetics. Broad-based management of WSLs includes methods for preventing demineralization as well as for encouraging remineralization of existing lesions. Successful preventive strategies include patient education, oral hygiene promotion, regular professional oral prophylaxis, and appropriate patient compliance.

For the noncompliant patient, appropriate preventive medicaments such as topical fluorides can aid in reducing demineralization.\(^3,4\) Since their introduction in the 1960s, fluoride varnishes have increasingly demonstrated their ability to reduce orthodontic-related enamel demineralization.\(^5\)–\(^7\) Varnish application is a preventive protocol that does not require patient compliance and also permits the orthodontist to benefit from the proven bond strength of composite resins. Prolonged contact time with fluoride varnish permits significantly more incorporation of fluoride than any other cooperation-based fluoride application systems.\(^3,4\) Apart from fluorides, casein phosphopeptide–amorphous calcium phosphate (CPP-ACP; Recaldent, Recaldent Pty) has been recently reported to possess topical anticariogenic effects, possibly by the incorporation of nanocomplexes into dental plaque and onto the tooth surface, thereby acting as a calcium and phosphate reservoir.\(^8,9\) There appears to be an inverse association between plaque calcium and phosphate levels and measured caries experience.\(^10\) Although studies on CPP-ACP have shown promising remineralization of already demineralized enamel, very few studies have documented its potential to prevent demineralization during orthodontic therapy.\(^11,12\) More recently, calcium sodium phosphosilicate (NovaMin, NovaMin Inc) has been reported to possess an anti-cariogenic and beneficial periodontal effect.\(^13\) The material, a bioactive glass originally developed for bone regeneration, is reactive when exposed to body fluids, depositing hydroxy carbonate apatite (HCA), a mineral that is chemically similar to the minerals in enamel and dentin.\(^14\) It has also been reported to reduce the viability of oral microorganisms, possibly by the alkaline nature of its surface reactions, which may reduce bacterial colonization in vivo.\(^15\)

The aim of this study was therefore to evaluate the effects of CPP-ACP (with and without fluoride) and calcium sodium phosphosilicate on enamel demineralization during orthodontic treatment and to compare the demineralization inhibition potential of these agents to that of a fluoride varnish that has already demonstrated considerable success in the prevention of orthodontically induced enamel demineralization.

**METHOD**

Seventy-five intact maxillary premolars extracted for orthodontic treatment were disinfected in 0.1% aqueous thymol solution for a week. The apical third of the root was sectioned off, and the teeth were secured to the inner surface of plastic lids of 10-mL specimen jars with adhesive wax (Fig 1). Teeth were then painted with a thin layer of acid-resistant nail varnish (Elle 18, Hindustan Lever Limited), creating an approximately 2 × 3-mm window of exposed buccal crown surface to simulate the position of an orthodontic bracket and its periphery. The nail varnish was allowed to dry for 20 minutes, followed by a reaplication that was allowed to dry overnight. The specimens were then randomly allocated to five different groups, one control and four different medicaments, of fifteen teeth each. Table 1 provides the details of the four different surface treatment medicaments used in the study.
Teeth in group 1 received no further treatment and served as the control; group 2 received a single application of Fluor Protector varnish; and groups 3, 4, and 5 received 0.5-mL applications of GC Tooth Mousse, SHY-NM paste, and GC Tooth Mousse Plus, respectively, according to the manufacturer’s instructions. All the specimens were then immersed in jars filled with 10 mL of constantly circulating ten Cate demineralizing solution (pH = 4.46) at room temperature.\textsuperscript{16,17} Every 4 hours the teeth were removed from the solution, rinsed with distilled water, and brushed with a medium-bristled toothbrush (Oral-B) without a dentrifice. GC Tooth Mousse, SHY-NM paste, and GC Tooth Mousse Plus were reapplied every 4 hours, whereas teeth in group 2 received only a single application of fluoride varnish during a total study period of 96 hours.

After 96 hours, all the teeth were removed from the solution, thoroughly rinsed with distilled water, and mounted in polyvinylchloride mounting rings with autopolymerizing acrylic resin. Buccolingual longitudinal sections 150 to 200 μm thick were obtained with a hard tissue microtome (Leica SP 1600, Leica Biosystems), soaked in distilled water, dried with absorbent paper, and evaluated under a trinocular research polarizing microscope (Olympus BX-51, Olympus) fitted with a three-chip charge-coupled device (CCD) camera (Proview, Media Cybernetics).

**Table 1** Details of surface treatment medicaments used in the study

<table>
<thead>
<tr>
<th>Product name</th>
<th>Active ingredient</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluor Protector varnish</td>
<td>0.92-mL ampoule contains 1 mg fluoride in form of fluorosilane</td>
<td>Ivoclar Vivadent</td>
</tr>
<tr>
<td>GC Tooth Mousse</td>
<td>Bioavailable CPP-ACP</td>
<td>GC Corporation</td>
</tr>
<tr>
<td>SHY-NM paste</td>
<td>Calcium sodium phosphosilicate (NovaMin)</td>
<td>Group Pharmaceuticals</td>
</tr>
<tr>
<td>GC Tooth Mousse Plus</td>
<td>Bioavailable CPP-ACPF and 0.2% w/w (900 ppm) of sodium fluoride</td>
<td>GC Corporation</td>
</tr>
</tbody>
</table>
Polarized photomicrographs were then made under maximum illumination and 10× magnification. Three specimens from the control group suffered a breakout during sectioning; therefore, 72 slides were obtained for final image analysis. The digital photomicrographs were analyzed with image analyzer software (Image-Pro Plus version 4.1.0.0, Media Cybernetics) that measures the linear distance from the tooth surface to its greatest and least demineralized regions and registers the largest and smallest values of depth of demineralization for each specimen (Fig 2).

**Statistical analysis and intra-operator error**

Descriptive statistics were calculated for all five groups using Excel 2007 (Microsoft). A one-way analysis of variance (ANOVA) test was used to determine statistically significant differences among the mean depths of demineralization for the five groups, followed by Bonferroni multiple comparison tests using SAS version 9.0 (SAS Institute) with a $P$ value < .05 being considered a statistically significant difference. All the procedures in the study were carried out by a single operator. Eight values from each of the five groups were recorded again after 20 days, compared with eight randomly selected values from the same group taken previously, and subjected to a paired $t$ test to assess the intra-operator error.
RESULTS

Intra-operator error
There was no significant difference in the values of depths of enamel demineralization when the readings were measured again after 20 days.

Descriptive statistics for depths of enamel demineralization
Descriptive statistics for the depths of enamel demineralization for all five groups are presented in Table 2. The mean (± SD) depths of enamel demineralization in different groups were found to be as follows:

- Group 1 (control): 141.7 ± 9.4 μm
- Group 2 (Fluor Protector): 59.1 ± 6.2 μm
- Group 3 (GC Tooth Mousse): 80.5 ± 3.1 μm
- Group 4 (SHY-NM): 77.9 ± 8.1 μm
- Group 5 (GC Tooth Mousse Plus): 71.1 ± 5.9 μm

Comparison of mean depths of enamel demineralization
ANOVA test indicated that there was a significant difference (P < .0001) in measurement depths amongst the various groups. The Bonferroni comparison tests demonstrated a statistically significant difference between the groups except between groups 3 and 4 and groups 4 and 5 (see Table 2).

DISCUSSION

The use of topical fluorides in various forms has been, to date, the most commonly used caries prevention protocol for the orthodontic patient. CPP-ACP and calcium sodium phosphosilicate have been introduced in recent years as supplements or substitutes for conventional fluoride-based systems. This study was undertaken to compare the effects of fluoride varnish, CPP-ACP (with and without fluoride), and calcium sodium phosphosilicate on enamel demineralization. Results of this study demonstrate that the greatest amount of enamel demineralization occurred in the control group (141.7 ± 9.4 μm), which was subjected to an artificial caries solution without the application of any medicament. The solution pH (4.46) and study period (96 hours) were intention-
Bichu et al

ally selected to limit the demineralization to a white spot lesion for accurate measurement of the lesion depth. These results imply that repeated exposures to an acidic environment in vivo as well could result in the development of such lesions within a short span of time.

The results also demonstrate that each medicament used in the study offered some enamel protection as compared with the control. The greatest protection against demineralization (mean depth, 59.1 μm) was seen in the group treated with Fluor Protector varnish. The ability of fluoride varnish to significantly inhibit enamel demineralization has already been documented. Fluoride varnishes have been found to remain in contact with enamel for several days due to their viscous nature, and Fluor Protector in particular has been noted to create a durable physical barrier between the enamel and cariogenic solution. Being a silane lacquer, it has very low viscosity and a good wetting action, allowing it to penetrate into the enamel porosities and mimic the tagging effect of composite resins.

The third group investigated in this study was treated with GC Tooth Mousse, a CPP-ACP formulation. Although the reduction in the demineralization by this agent (mean depth: 80.5 μm) was lesser than the fluoride varnish, the measured depths were still significantly lower as compared with the control. This is in accordance with other studies that have demonstrated significant reductions in enamel demineralization using CPP-ACP preparations in vitro.

No study hitherto has evaluated the effect of calcium sodium phosphosilicate (NovaMin) on enamel demineralization during orthodontic treatment, although preliminary in vitro investigations on the remineralization ability of NovaMin-containing dentrifices have been conducted. In this study the fourth group was treated with SHY-NM paste (a NovaMin formulation), and the results demonstrate significant reductions in the depths of enamel demineralization (mean depth: 77.9 μm) as compared with the control. The ability of this paste to inhibit demineralization was less than that of the fluoride varnish but comparable to the effect of GC Tooth Mousse. These results are in accordance with another study that utilized scanning electron microscopy and energy-dispersive x-ray spectroscopy for analysis of teeth subjected to demineralization cycles and concluded that toothpastes containing Recaldent (CPP-ACP) and especially NovaMin have the potential to remineralize enamel. Thus, along with CPP-ACP, calcium sodium phosphosilicate could serve as a supplement or alternative to fluoride for the control of demineralization.

The comparison of the measurement depths in this study thus served as an indicator of the demineralization inhibition potential of the different agents.
Fluoride varnish is a professionally applied medicament; this coupled with its transparent and esthetic appearance makes it well suited for the control of demineralization in the orthodontic patient. The utility of varnishes, however, has been questioned in situations where decalcification has already occurred. Also the occurrence of fluorosis associated with high fluoride concentrations may act as a deterrent to their use. CPP-ACP and calcium sodium phosphosilicate may be indicated as supplements or substitutes for fluoride during orthodontic treatment. They may also be utilized when the chances of fluorosis are significant or where varnish is contraindicated due to gingival inflammation or contact allergy. A potential drawback of these agents is that they are patient-dependent. A patient who does not follow oral hygiene and diet instructions is likely to be noncompliant for the use of these medicaments as well. These formulations have also been postulated to cause remineralization of existing lesions and may be utilized in the management of posttreatment lesions.

A potential drawback of this study is the inability to take into consideration the protective potential of the salivary pellicle. The salivary pellicle is thought to act as a diffusion barrier or permselective membrane on the enamel surface providing protective effects against remineralization from various acidic challenges in the oral cavity. The absence of the pellicle on the teeth, especially those in the control group, could possibly accentuate the advent and the extent of demineralization noted in the study. Substantiation of the findings of this in-vitro study is therefore necessary by undertaking clinical trials.

CONCLUSION

Within the limitations of this in vitro study, the following conclusions can be drawn:

- Significant enamel demineralization was found to occur in the control group that consisted of teeth subjected to a cariogenic solution without exposure to any medicament.
- This demineralization could be effectively reduced by the application of various surface treatment agents used in the study.
- The agents, arranged in the descending order of their efficiency, are: Fluor Protector varnish, GC Tooth Mousse Plus, SHY-NM paste, and GC Tooth Mousse.
- Use of such topical medicaments could prove to be beneficial for patients undergoing orthodontic treatment and who are at a risk of developing enamel decalcification.
- Clinical trials investigating the effectiveness of these surface treatment agents during orthodontic treatment would be required to confirm these in vitro results.

ACKNOWLEDGMENTS

The authors would like to sincerely acknowledge the guidance provided by Dr Suniel Sunny, Professor & Head, Department of Orthodontics, Annoor Dental College and Hospital, Kerala, India and Prof Dr K. Sadashiva Shetty, Professor & Head, Department of Orthodontics and Dean, Bapuji Dental College and Hospital, Davangere, India, during the conduct of the study.
REFERENCES


