"Prevention of enamel demineralization around orthodontic brackets – An in vitro study"

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Abstract
Introduction: Despite extensive research in various preventive technologies over the years, white spot lesion (WSL) development in association with orthodontic treatment with fixed appliances remains an unwanted clinical problem.

Materials and Methods: This study was conducted to compare the efficacy of various cariostatic products available commercially that would help in prevention of white spot lesions. Total 120 healthy extracted premolars were taken and divided into a sample of four groups. GC Tooth Mousse, Clin pro and Anticay were compared for their protective efficiency. Three test groups had three different topical agents applied and the fourth group acted as a control. After the test materials were applied they were subjected to pH cycling using artificial saliva with a pH of 4.5 that acted as a demineralization agent and fluorescence was assessed using spectroscopy.

Results: One way Anova and Student’s t’ test revealed the efficacy of all the three materials as cariostatic agents, GC Tooth Mousse and Anticay were revealed to have the best protective efficiency.

Conclusion: GC Tooth Mousse and Anticay can be used by the orthodontic patients as an in home application product for the prevention of development of white spot lesions.

Keywords: White spot lesions, Spectroscopy, Artificial Saliva, CPP-ACP, CaSP.

Introduction

Demineralization around orthodontic brackets is a common problem during fixed appliance therapy when oral hygiene is poor. Lesions become first clinically visible as white spots, due to an optical phenomenon that is caused by mineral loss in the surface or sub-surface enamel.1,3 Previous studies on the mechanical and crystallographic characteristics of these incipient carious lesions have shown that there is a 10-50% reduction in mineral content.1,3 White spot lesions (WSLs) have been previously reported to develop within 4 weeks of band/bracket placement. The prevalence of WSLs in orthodontic patients has been reported in the range of 50-96%.2,5,6 The increased prevalence of enamel demineralization is largely due to patients inability to perform effective oral hygiene measures in the presence of brackets and to the increased plaque retention around orthodontic attachments.1,3

Many published studies and review articles advocate management of orthodontic WSLs with preventive strategies that include patient education, routine professional prophylaxis, and appropriate preventive medicaments such as topical fluorides.1,3-11

The three products GC Tooth Mousse (Caesin Phosphopeptoid – Amorphous Calcium Phosphate), Clin proTM (Sodium Fluoride 1.1%), Anticay (Calcium Sucrose Phosphate) show promise in their ability to prevent enamel demineralization during orthodontic treatment, however clinical data comparing the efficacy of these three cariostatic products is lacking. Present article describes the effect on demineralization, of various topical agents available commercially which claim to reduce demineralization when applied topically adjacent to orthodontic brackets using quantitative evaluation methods.

Material and Methods

The study was conducted in the Department of Orthodontics and Dentofacial Orthopaedics, Seema Dental College and Hospital, Rishikesh, Uttarakhand and Indian Institute of Technology, Kanpur, Uttar Pradesh.

One hundred and twenty sound, human premolars free from any white spot lesion extracted for orthodontic reasons were collected and stored in 10% buffered formalin solution. Teeth with hypoplastic area, cracks or gross irregularities of enamel surface were excluded from the study. The extracted teeth were rinsed and stored in de-ionised water to prevent dehydration and bacterial growth before the experimental use (Fig. 1a). After surface preparation, the liquid primer Transbond XT (3M-Unitek) was applied to the etched surface and cured for 10 seconds. All the brackets - (3 M Unitek, Victory series) were bonded to the teeth using an adhesive - Transbond XT (3M-Unitek). Then the bonding adhesive was light cured for 10 seconds on each side of the bracket using a light emitting diode (L.E.D) curing unit (SDI-Radius Plus).

After bonding, 2mm window was created encircling the brackets - by applying nail polish varnish on rest of the tooth surface except for the 2 mm area surrounding the bracket margins. (Fig. 1b) The
following cariostatic agents were utilized for comparison: 1) GC tooth mousse (casein phosphopeptide amorphous calcium phosphate complexes) 2) Clin pro 1.1% (NaF) 3) tooth min (Anticay) (calcium sucrose phosphate) as shown in Fig. 2.

Fig. 1: Tooth surface a) prior to bonding b) after bonding and creation of window

The sample was then divided into four groups containing 30 teeth each. (Table I)

| Group A | Group B | Group C | Group D |
|---------|---------|---------|---------|
| Test material ‘A’ applied around the bracket margins. | Test material ‘B’ applied around the bracket margins. | Test material ‘C’ applied around the bracket margins. | Control with no test solution placed around the bracket margins. |

Fig. 2: Test materials – GC tooth mousse, clinpro, tooth min

After bonding mean fluorescence of each tooth was calculated before the application of any test material with the help of fluorolog III (Spectroscopy) and the base line readings were recorded for each tooth for further comparisons. This study was a double blind study and after baseline measurements the test solutions were applied. Artificial caries like lesions were created in the exposed enamel by suspending the teeth in an artificial saliva prepared in the Biochemistry lab of Seema Dental College and Hospital Rishikesh. In order to simulate the oral conditions a strongly high cariogenic challenge was reproduced using an Artificial saliva with a pH adjusted to 4.5 as the demineralization solution, seeking an ionic balance and more similarity to the dynamics occurring in the oral cavity, particularly because it has been shown that fluoride release in artificial saliva is slower than it is in water. The composition of the artificial saliva was similar to the standard salivary substitutes available. Lactic acid was used to adjust the pH at 4.5.

Table 2: Composition of artificial saliva

| Reagent               | Concentration | Reagent               | Concentration |
|-----------------------|---------------|-----------------------|---------------|
| Sodium carboxymethyl cellulose | 10 g/l        | Di–potassium hydrogen orthophosphate | 0.80 g/l |
| Potassium chloride    | 0.62 g/l      | Potassium di hydrogen orthophosphate | 0.30 g/l |
| Sodium Chloride       | 0.87 g/l      | Sodium fluoride       | 0.0044 |
| Magnesium Chloride    | 0.06 g/l      | Sorbitol              | 29.95 |
| Calcium Chloride      | 0.17 g/l      | Methyl p-hydroxy-benzoate | 1.00 |

The experimentation lasted till 96 hours. All specimens were immersed in 10 mL of the demineralization solution for 96 hours at room temperature, with the solution changed every 4 hours. At 4 hourly intervals, all specimens were rinsed with deionised water, blotted with paper tissues and air dried for approximately 2 minutes. The test material was reapplied and the demineralization solution was changed till a total of 96 hours. (Fig 3) Fluorolog III (spectroscopy) readings were taken for each tooth at the end of 96 hours. (Fig. 4). The difference in fluorescence
(change F) between baseline and 96 hours was then calculated.

The observations / results thus obtained were subjected to student’t’ test, one way Anova and Tukey post hoc test.
At the end of 96 hours the mean change in fluorescence recorded were - Group D (\(\% F = 71.48\)) ; Group A (\(\% F = 20.24\)) ; Group B (\(\% F = 36.97\)) and Group C (\(\% F = 25.81\)) ; (Anova P = < 0.01 ).(Fig 5 & 6 ) Control D delta F and \(\% F\) were significantly greater when compared to all test materials. Statistically significant differences were observed in all test groups with P<.001 for all the three tested medications when compared with the control D. The topical application of GC Tooth Mousse and Anticay reduced the delta F when compared with the control D samples P < .001. (Tukey post hoc test) (Fig. 7) The comparison amongst all the groups were significant; the mean change in fluorescence and the proportional change amongst group A and C was however not significant.

### Table 3: Mean Delta F

|               | MEAN     | STDEV   |
|---------------|----------|---------|
| GROUPA (GC TOOTH MOUSSE) | 17128    | 18261.41 |
| GROUP B (Clin pro TM)     | 31191    | 14892.52 |
| GROUP C (Anticay)         | 21811.33 | 19315.8  |
| GROUP D (CONTROL)         | 59859.33 | 27586.87 |

The mean Delta F - difference in intensity at baseline and end of experimentation within each group was calculated using the formula, \(\% F = (\_F\) baseline – \(_F\) 96hr)/ (\_F baseline _ 100\%

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**Results**

The results of the study showed a significant reduction in delta F for Group A and C compared to Group B and Group D.

**Fig. 3:** Teeth dipped in artificial saliva

**Fig. 4:** a) SpectrAcq controller & set up b) Measurement of individual tooth fluorescence

**Fig. 5:** Graph showing the comparison of pre and post emission spectra of a) group A - GC Tooth Mousse; b) group B - Clin Pro

**Fig. 6:** Graph showing the comparison of pre and post emission spectra of a) group C -ToothMin b) group D – Control
Discussion

White spot lesion (WSL) is a common iatrogenic effect seen in patients undergoing orthodontic treatment with fixed appliances.

It is quintessential for clinicians to develop preventive measures as soon as appliances are placed. Previous studies have shown that fluoride or ACP containing materials have a potential to minimize demineralization. Volker was the first to show that topical application of fluoride prevents enamel solubility. Fluoride can be incorporated into the crystalline lattice of the mineral of dental hard tissues, resulting in a mineral phase (fluoroapatite) that is less soluble and more acid resistant.

Our findings suggest that the topical application of GC Tooth Mousse offered the best protection against demineralization which is similar to other published previous studies. The mean demineralization of the control group also corresponds with the previous studies. Although some studies have reported NaF to provide a similar protection as Tooth Mousse, our findings do not confirm with that. In our study significant difference exists between these two groups in terms of their cariostatic ability. The possible reason for this variation is use of a purely chemical remineralization agent in other studies (Carbopol), in which the oral conditions have not been taken into consideration.

In this study special care has been taken to simulate the oral conditions by usage of an artificial saliva solution with the pH adjusted to 4.5 as the demineralization solution so that the shortcomings of the in-vitro set up could be overcome. Also another material – Anticay – Calcium Sucrose Phosphate was tested for its effectiveness as the literature present on this material was relatively less.

In accordance with the findings it can be concluded that both GC Tooth Mousse and Anticay can be used by the orthodontic patients as an in home application product for the prevention of development of white spot lesions.

The protective effect of GC Tooth Mousse and Anticay has been proved however further research is needed to determine the efficacy of Anticay in in-vivo conditions and also to assess the mode and duration of application of any of the above mentioned test material that would best help to prevent enamel demineralization or in other words would help to combat the unaesthetic side effect or the undesired stigma of orthodontic treatment – WHITE SPOT LESIONS.

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Conclusion

The use of Tooth Mousse and Anticay significantly helped to prevent the demineralization. Clin pro is an effective remineralizing agent but its effectiveness is less as compared to GC Tooth Mousse and Anticay. Artificial saliva at a pH of 4.5 produced subsurface lesions similar to three months intra oral pH cycling.

Thus we recommend the use of GC Tooth Mousse / Anticay for all orthodontic patients to provide protective effect against demineralization and potentially remineralize subclinical enamel demineralization if present.

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