University J Dent Scie 2023; Vol. 9, Issue 4

Prevention of Enamel Demineralisation Around Orthodontic Brackets (An In-vitro Study)

Abstract:

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

Aims and objectives: The present study is aimed at determining the difference between demineralisation of enamel at normal and reduced pH around orthodontic brackets and to compare the efficacy of various remineralisation agents used in the study thus establishing the most reliable product available commercially for in-home application that would help in secondary prevention of white spot lesions.

Material and methods: The study sample used for this study comprised of 50 healthy, caries-free premolars that were free from any enamel defects. The teeth were extracted and stored in 10 % buffered formalin till the study. All the teeth were bonded and divided into five groups with 10 teeth each and after application of different cariostatic agents according to the pre-decided criteria were subjected to pH cycling using artificial saliva with a pH of 4.5 as the demineralization agent in order to simulate the oral environment in the in-vitro study. The teeth were analysed using Field-emission Scanning Electron Microscopy (FE-SEM).

Results: The use of Anticay and Amflor significantly helped to prevent the demineralization. Stim Ri-namel was found to be an effective re-mineralizing agent but its effectiveness is less as compared to Anticay and Amflor. Artificial saliva at a pH of 4.5 produced subsurface lesions similar to 3 month intra oral pH cycling.

Key-words: Stim-Ri-namel, Amflor, Toothmin

Introduction:

The increase in enamel demineralization can be attributed in part to increased plaque around orthodontic brackets because of increased difficulty of plaque removal as well as increased bacterial adhesion to composite resin bonding materials.[1]

Much research is now being focused upon reducing the occurrence of decalcification during orthodontic treatment. Researchers have turned their attention toward appliance design, bonding materials, use of fluorides, sealants and improving the quality of remineralising agents.

With the availability of various remineralizing agents, viz. Amflor, toothmin, Ri-namel etc a need for meticulous comparison becomes desirous as it has never been done in previously reported studies. Thus, this study aims to :

1) Determine the difference between demineralisation at *normal and reduced pH* around orthodontic brackets.
2) Compare the efficacy of remineralisation agents used in the study for commercial purposes.

Access this article online

Website: www.ujds.in

DOI: https://doi.org/10.21276/ujds.2023.9.4.20

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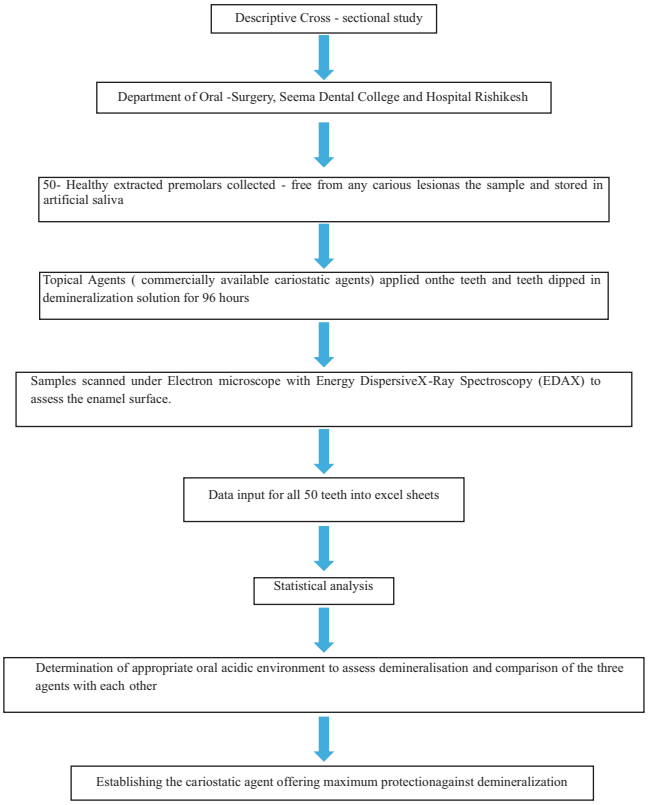
Received : 12 March, 2022, **Published :** 30 November, 2023

How to cite this article: Saksham Duseja, Narayana Prasad, Tarun Sharma, Tarun Rana, Anupa Rawat, & Prem Prakash. (2023). Prevention of Enamel Demineralisation Around Orthodontic Brackets (An In-vitro Study) . UNIVERSITY JOURNAL OF DENTAL SCIENCES, 9(4). 97 - 104

Materials and Methods:

The study was conducted in the Department of Orthodontics and Dentofacial.

Orthopedics, Seema Dental College and Hospital, Rishikesh, Uttarakhand and the following methodology was used to proceed with it.



Study Design

Sample size estimation:

Sample size estimation was done by using GPower software (version 3.0). Sample size was estimated for one way ANOVA test. A minimum total sample size of 48 was found to be sufficient for an alpha of 0.05, power of 80 % and 0.65 as effect size.

Preparation of Sample:

After extraction the collected teeth were rinsed and stored in Artificial Saliva at pH 7.0 to prevent dehydration and bacterial growth before the experimental use. This was followed by conventional method of bonding of brackets after which about 2mm window was created around them. Nail paint on rest of the tooth surface except for the 2 mm area surrounding the bracket margins was applied as shown in the figure.

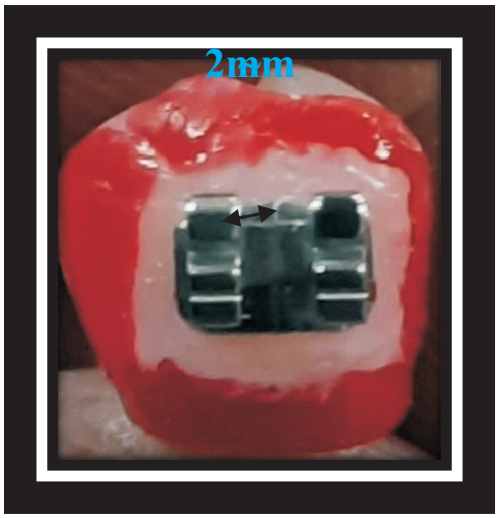


Figure 1 : Preparation of Sample

The teeth were then divided into 5 groups containing 10 teeth each. The 5 groups were:

I) Samples immersed in artificial saliva at a pH of 7.0

II) Samples immersed and kept at a salivary pH of 4.5

III) Those which had bracket that had test material 'A' (Toothmin) applied around the bracket margins and immersed in artificial saliva of pH 4.5

IV) Those that had test material 'B' (Stim- Ri-namel) applied around the bracket margins immersed at a salivary pH of 4.5

V) Those that had test material 'C'(Amflor) applied around the bracket margins immersed in pH 4.5

Inclusion Criteria:

1. The teeth were free from white spot lesions and other enamel defects.
2. Buccal surface of the teeth was kept intact

Exclusion Criteria:

1. Periodontally compromised teeth
2. Endodontically compromised teeth

The study was designed as a double blind one, in which the observers and the performer were unaware of the test solutions that were being applied in order to eliminate selection bias. The efficacy of Toothmin toothpaste has been studied elaborately hence in order to eliminate bias among the commercial products, all the test materials were disposed in three different identical containers in randomized fashion and these containers were labelled as A,B, C corresponding to sample numbers 3, 4 and 5 respectively.

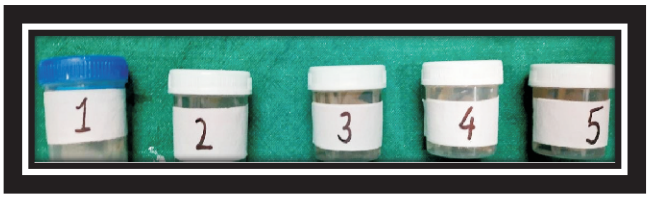


Figure 2: Distribution of Samples

After application of topical agents on the prescribed buccal surface of tooth samples mentioned above- specimens were immersed in demineralization solution at room temperature and the room temperature was maintained using a laboratory incubator (Sesw Lab Supplies, India).

Figure 3 : Laboratory Incubator

The artificial saliva was prepared in the Biochemistry laboratory of the college itself using the method described by Preetha et al[23] (Fig. 4)

Figure 4 : Preparation of Artificial Saliva[23]

In order to simulate the oral conditions a strong cariogenic challenge was reproduced using artificial saliva with pH 4.5 adjusted with lactic acid (Fig 5)

Figure 5 : Adjustment of pH – monitored using a Digital pH meter

The present study was conducted using artificial saliva, seeking an ionic balance and more similarity to the dynamics occurring in the oral cavity. The composition of artificial saliva prepared is as follows :

Table 1 : Composition of Artificial Saliva

De-ionized Water	1 L
Potassium Chloride	0.62g/l
Sodium Chloride	0.87g/l
Magnesium Chloride	0.06g/l
Calcium Chloride	0.17g/l
Di-Potassium Hydrogen Orthophosphate	0.80g/l
Potassium Di-hydrogen orthophosphate	0.30g/l
Sodium Fluoride	0.0044g/l

***Note** :- pH 7.0 & pH 4.5 of artificial saliva were adjusted with dilute acetic acid and lactic acid, respectively.

After bonding, the sample was prepared for investigation via Field emission-Scanning Electron Microscope using EDX-EBSD system (Quanta 200F FEI, Neatherlands). (Fig 6)

Figure 6 : Field emission-Scanning Electron Microscopy with EDX-EBSD system

The root portion was eliminated from the cementoenamel junction using a carbide cutting disk (Rapido-Cut Mounted Diamond Disc 180mm, Germany). After that the tooth samples were placed on stubs held by glue stickers and transferred to the platform component of the EDX-EBSD system to assess the enamel surface. (Fig 7)

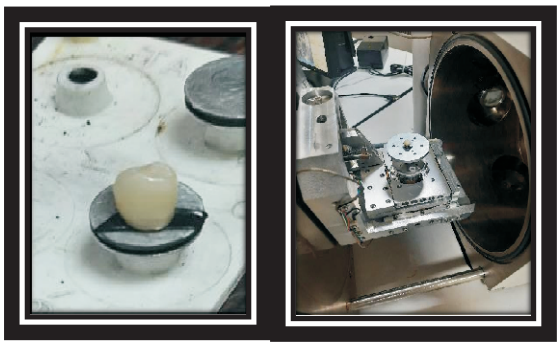


Figure 7: Activation of Sampling Assessment

Surface images of the sample for all the groups were thereafter scanned at X1000.

Method of Evaluation:

The obtained SEM images were used to evaluate the efficacy of remineralization around the orthodontic attachment by scoring the roughness of enamel surface.

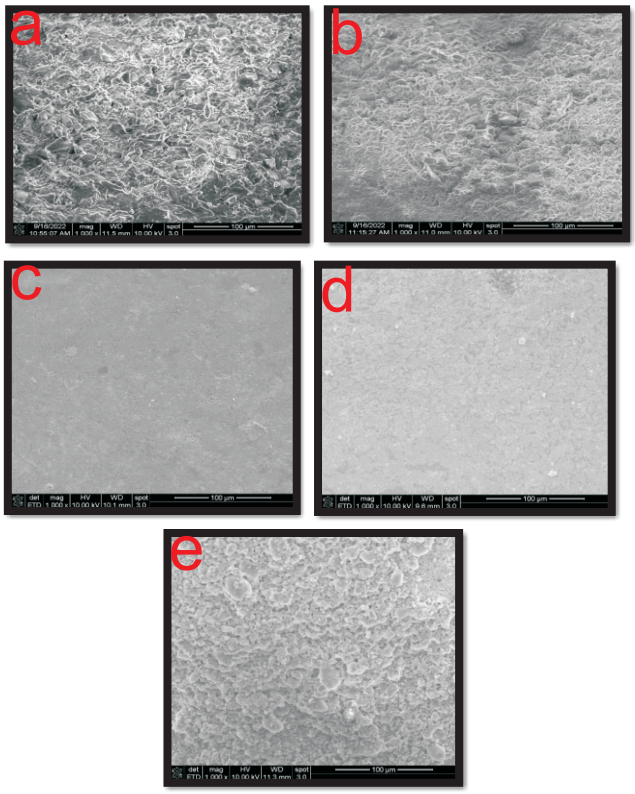


Figure 8: (a-e) Scanned images depicting various degrees of surface roughness

All the SEM pictures were scored from 0 to 5, based on comparative surface roughness by two examiners at three different time intervals with a difference of 4 days between each scoring. (Joshi et al)[2]

Then, the scores were graded as follows:

0 and 1—mild roughness

2 and 3—moderate roughness

4 and 5—severe roughness

Statistical procedures were carried out in 2 steps :

1. Data compilation and presentation
2. Statistical analysis

Data Compilation and Presentation :

The data was then compiled systematically. The tables were prepared in an excel spreadsheet and the total data was subdivided and distributed meaningfully. The statistics were applied and results obtained were structured in a tabulated and graphical manner.

Statistical Analyses:

Statistical analyses were performed using a personal computer with Statistical Package for Social Sciences software (SPSS version 22.0). Datacomparison was done by applying specific statistical tests to find out thestatistical significance of the obtained results. Nature of the data was checked using Kolmogorov Smirnov test and Shapiro wilk test. Probability value of 0.05 or less was considered as statistical significance.

It was found that data was not normally distributed hence non-parametric tests of significance were applied in the present study.

1. Kruskal Wallis H test Analysis of variance was applied for multiple group comparison and,
2. Mann Whitney U test was performed since significant difference was found between the groups.

Method Error:

To assess inter-observer error all the SEM images were scored by the other observer three times at the interval of 4 days to eliminate the method errors calculated by Intraclass and Interclass Correlation Test. This shows agreement between scorings done by Examiner 1 and Examiner 2 which rules out inter-examiner error of measurement for both single measures and average measures.

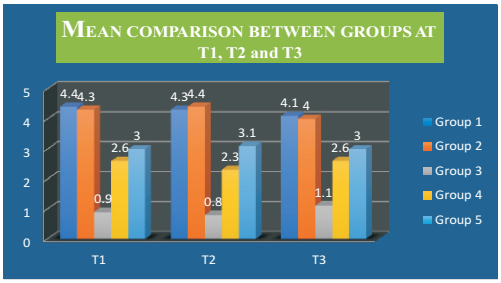
Results :

The clinical scanning electron microscopic study was conducted under the aegis of the department of Orthodontics and Dentofacial Orthopaedics, Seema Dental College and Hospital, Rishikesh in collaboration with Indian Institute of Petroleum, Dehradun, Uttarakhand.

TABLE 1: Mean Comparison Between Groups at T1, T2 And T3 using Kruskal Wallis ANOVA

	Group	*Mean*	*Std. Deviation*	*Chi Square*	*p value*	*Post hoc*
T1	Group A (Normal ph)	4.4	0.52	39.38	0.001**	A, B>D, E>D, C
	Group B (4.5 without toothpaste)	4.3	0.48			
	Group C (4.5 with toothmin)	0.9	0.32			
	Group D (4.5 with Ri- namel)	2.6	0.97			
	Group E (4.5 with Amflor)	3	0.67			
T2	Group A (Normal ph)	4.3	0.48	41.463	0.001**	A, B >D, E> D, C
	Group B (4.5 without toothpaste)	4.4	0.52			
	Group C (4.5 with toothmin)	0.8	0.42			
	Group D (4.5 with Ri- namel)	2.3	0.82			
	Group E (4.5 with Amflor)	3.1	0.57			
T3	Group A (Normal ph)	4.1	0.74	35.687	0.001**	A, B >D, E> D, C
	Group B (4.5 without toothpaste)	4	0.67			
	Group C (4.5 with toothmin)	1.1	0.57			
	Group D (4.5 with Ri- namel)	2.6	0.70			
	Group E (4.5 with Amflor)	3	0.67			

Overall comparison was done between all groups at different time intervals. The results show statistically significant difference between all five groups in Time interval (T1, T2 and T3) with Chi square value of 39.38, 41.46 and 35.68 at a p value of <0.05. Post hoc comparison showed no significant difference between group 1 and group 2 in all Time intervals at a p value of <0.05, whereas, significant difference was seen in Group A,B and Group C, Group A,B and D and Group A,B and E at all time intervals i.e. Group A and B show a higher mean rank value than Group C, D andE suggesting a reduction in surface roughness of enamel upon exposing it to Toothmin, Ri-namel and Amflor Remineralising toothpaste in comparison to exposing it to plain solutions of pH 7.0 and 4.5. (Table 1, Graph 1)



GRAPH 01: Mean Comparison Between Groups At T1, T2 And T3

	Group	*Mean Rank*	*Sum of Ranks*	*Z value*	*p value*
T1	1	11	110	-0.457	0.648
	2	10	100		
T2	1	10	100	-0.457	0.648
	2	11	110		
T3	1	10.9	109	-0.336	0.737
	2	10.1	101		

TABLE 2: Mean Comparison Between Group 1 And Group 2 At Different Time Intervals using Mann Whitney U Test

Table 02 shows individual comparison between group 1 and group 2 at different time intervals (T1, T2 and T3). The results show no significant difference between group 1 and group 2 in all Time intervals at a p value of <0.05. This indicates no significant decrease in surface roughness of enamel upon exposing the surface with a pH of 4.5 as compared to normal pH of 7.0.

Discussion :

It has been observed clinically that orthodontic patients develop significantly more WSL's than non-orthodontic patients. This demineralization of enamel is due to the decrease in oral pH because acidogenic bacteria in the dental plaque produce acids while metabolizing carbohydrates.[3]

Tufekci et al in 2011 stated that the development of white spot lesions (WSLs) is attributed to prolonged plaque accumulation around the brackets. Not only do the fixed orthodontic appliances (brackets and bands) make conventional oral hygiene procedures more difficult, they also increase the number of plaque retention sites on the surfaces of the teeth that are normally less susceptible to caries development.[3]

Orthodontic treatment with complex loop designs further increases the risk for development of WSL due to the creation of additional retention sites on surfaces generally not susceptible to caries.Hence a strong co-relation exists betweenoral hygiene and caries incidence in orthodontic patients as compared to non orthodontic individuals.[4] Other important factors in the development and progression of carious lesions are the patient's modifying factors, including medical history, dental history, diet, levels of calcium, phosphate, bicarbonate in saliva, fluoride levels and genetic susceptibility[5].

The incipient carious lesions represent earliest phase of tooth decay or demineralization and are capable of being reversed, arrested or progression to cavitation.

Several studies have reported a significant increase in the prevalence and severity of demineralization after orthodontic therapy compared with controls, and overall prevalence amongst orthodontic patients ranges from 2 to 97 percent.[6,7-9]

Various techniques have been proposed in literature for the prevention of demineralization and the treatment of lesions after debonding to restore the esthetics in the best possible way.[10,11] Hence taking into consideration the limitations of various techniques mentioned in the literature, the present study was done using three commercially available remineralizing agents : Toothmin toothpaste (Calcium Sucrose phosphate), Amflor remineralizing active toothpaste (Amine fluoride) and Stim Ri-namel (Vitamin E rich fluoride)[12].

The potential for topical fluoride application in caries prevention program is well recognized. Volker[12] was the first to show that topical application of fluoride prevents enamel solubility. Clinical studies have demonstrated a highly significant reduction in the incidence of dental caries following the topical application of the tooth surfaces with fluoride solution. A variety of fluoride forms other than solutions have been tried by many authors, to determine the most effective form, chemical or physical, of decreasing the enamel surface demineralization.

Shen et al[13] and Reynolds[14] compared the enamel remineralization ability of mouth rinse containing CPP-ACP with that of mouth rinse containing fluoride in an intraoral enamel demineralization model. They demonstrated that the mouth rinse containing 0.4% CPP-ACP and 220 ppm F produced 19% enamel subsurface lesion remineralization compared with 8 % remineralization by the 220 ppm F rinse and 14% remineralization by 0.4% CPP-ACP rinse. The results supported the role of fluoride in promoting remineralization and demonstrate an important facilitation of the effect of fluoride by CPP-ACP.

Anticay (Calcium Sucrose Phosphate) reduces the rate of demineralization of enamel and increases the rate of remineralization by a common ion effect[17] .It provides both calcium and phosphate ions in a soluble form at high concentrations – that states to have a cariostatic effect. The sucrose phosphate anion adsorbs directly onto the enamel surface, thereby inhibiting the process of demineralization[18]. It also actively neutralizes plaque acids[19] – thus may be an effective option in the management of white spot lesions.[20]

Stim Ri-namela Vitamin E rich fluoride remineralising toothpaste has never been experimented before for its cariostatic ability under the clinical conditions. Hence this study was undertaken with an aim to compare the clinical effectiveness of the three previously described cariostatic agents and thus to establish and conclude the best in home application method for prevention of enamel demineralization.

Before experimentation all the teeth were removed from the storage solution, washed with double de-ionized water and surface dried using air spray from a three-way syringe. All the debris was removed with Ultrasonic Scaler (UDS-J, Woodpecker)[16] and then washed with double de-ionized water before placing the dried teeth into an autoclave. Bonding was done on each premolar tooth – Brackets (Ormco-Mini Diamond™) were placed after etching the enamel suface for 60 seconds[21] and adhered with light cured resin composite – Enlight™ (Ormco) and cured with L.E.D curing light for 20 seconds[22] (SDI Radii Plus – 430 - 480 nm . 1000 mW intensity.)

After bonding of the brackets a 2mm window surrounding the brackets was created. Nail polish varnish was applied on the entire tooth surface except for a 2mm area surrounding the bracket surface – hence only this window was susceptible to the acid attack.

The teeth were then divided into five groups comprising of ten teeth each that formed the study sample.

In all the previous studies done to assess the prevention of demineralization, the demineralization medium was prepared using the composition of a particular salivary substitute. The demineralization solution contained 1 Litre of De-ionized water with various chloride salts and phosphate compounds enriched with sodium fluoride[23]

However, data was lacking regarding the efficacy of various cariostatic agents under the clinical conditions thus in order to simulate the acidic oral conditions an Artificial saliva with a pH adjusted to 4.5 was used as the demineralization solution[24].

All specimens were immersed in 10 mL of the solution for ninty six hours at room temperature, with the solution changed every six hours. Enamel crowns from groups 3, 4 and 5 were immersed in the demineralization solution with exposure to the topical solutions as mentioned above, enamel crowns from group 2 was not treated with any solution before immersion in the demineralization solution of pH 4.5. Group

1 was immersed in artificial saliva with pH of 7.0. At six hourly intervals, all enamel specimens were rinsed with deionised water, with topical solutions reapplied to the specimens and the demineralization solution changed.

At the end of ninety six hours, all enamel specimens were rinsed with de-ionised water, blotted with paper tissues, and air dried for approximately two minutes. The specimens were then re-immersed in artificial saliva at a normal pH and subjected to SEM study after sectioning the root and removing it from the sample with a carbide cutting disk[15]. The lingual cusps were flattened-off to place them over the stubs before placing the sample under the scanning electron microscope. The sample was placed at 10mm distance perpendicular to the X-ray beam projector for the rough surfaces to be detected efficiently.

The limitation of this study was that though maximum efforts were taken to simulate the oral conditions yet the basic underlined differences between an in – vitro study test model and an in-vivo design cannot be completely ruled out thus future endeavours to establish the potency of these materials under in vivo conditions especially Ri-namel – that is a relatively newer product and literature is lacking on its efficacy – must be undertaken in order to assess the mode and duration of application of these products that would help the patient to gain the maximum protection against WSL's.

Conclusion :

Within the limitations of this in vitro study, the following conclusions were drawn.

1. Topical application of all the three cariostatic agents – Toothmin toothpaste, Stim Ri-namel and Amflor was effective in preventing enamel demineralization.
2. When compared to Stim Ri-namel, Toothmin toothpaste offered better protection against the acidic challenge in vitro.

Stim Ri-namel's ability in preventing demineralization was similar to that of AmflorRemineralising toothpaste indicating the insignificant effect of adding Vitamin E to fluorides.

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