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## RESEARCH ARTICLE

# ASSESSMENT FOR THE EFFICACY OF VARIOUS REMINERALISING AGENTS DURING FIXED ORTHODONTIC MECHANOTHERAPY: *IN VIVO* STUDY

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**ABSTRACT**

**Introduction:** Topical agents GC Tooth Mousse and Toothmin toothpaste show promise in their ability to prevent enamel demineralization during orthodontic treatment, however clinical data comparing the efficacy of these remineralizing is lacking. Therefore, the purpose of this in – vivo study was to investigate the efficacy of remineralizing agents available commercially which claim to reduce demineralization when applied topically adjacent to orthodontic brackets. Materials and **Methods:** The total study sample comprised of sixty sound premolar teeth from thirty subjects that will undergo fixed orthodontic mechanotherapy involving extraction. Data collected by scoring of the SEM images. The clinical scanning electron microscope study includes comparison of individual right side (subgroup a) and left side (subgroup b) Group (Control Group) along with overall comparison of statistics with each other (Group 1 and Group 2).

**Results:** It was observed that the topical application of GC Tooth Mousse , Group 1 subgroup a and Toothmin toothpaste, Group 2 subgroup a was associated with a significantly statistical variation in the reduction of mean surface roughness when compared with the control Group 1 subgroup b , Group 2 subgroup b samples indicating that both GC Tooth Mousse and Toothmin toothpaste can be used by the orthodontic patients as a remineralising agent for the prevention of development of white spot lesions.

**Conclusion:** The ability of Toothmin toothpaste in preventing demineralization was similar to that of GC Tooth Mousse. Topical application of the remineralizing agents – GC Tooth Mousse and Toothmin toothpaste was effective in preventing enamel demineralization.



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## INTRODUCTION

Enamel demineralisation is an undesirable but common complication of orthodontic fixed appliance therapy. 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. 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. White spot lesions have been previously reported to develop within 4 weeks of band/bracket placement. A review of literature in 2005 reported 2% to 97% prevalence for white spot lesions associated with orthodontic treatment. It has been previously shown that patients with fixed orthodontic appliances have increased levels of acidogenic bacteria present in plaque, most notably *S. Mutans* and *Lactobacilli.*

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The elevated levels of bacteria are responsible for decreasing the pH of plaque in orthodontic patients more than that of non-orthodontic patients. The plaque layer on the enamel surface provides a source of acid production as the bacteria produce hydrogen ions from the metabolic breakdown of fermentable carbohydrates. As hydrogen ions are released, the acids diffuse through the plaque into the adjacent enamel. Once enamel is exposed to a critical pH around 5.5, it begins to dissolve causing demineralization of hydroxyapatite tooth structure.[10] The layer of plaque also acts as a physical barrier by limiting the diffusion of acid away from the tooth surface. Therefore, the potential for remineralization from the available exogenous calcium and phosphate ions in the patients' saliva is greatly reduced in the presence of plaque. The resulting demineralization and prevention of remineralization leads to the development of white spot lesions that often persist and cause long term esthetic problems. Many published studies and review articles advocate management of orthodontic white spot lesions with preventive strategies that include patient education, routine professional prophylaxis, and appropriate

preventive medicaments. GC Tooth Mousse (Casein Phosphopeptide Amorphous Calcium Phosphate complexes) (CPP-ACP) is also claimed to exhibit remineralizing ability. The proposed anticariogenic mechanism for CPP-ACP is the localization of ACP at the tooth surface which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel reducing demineralization and enhancing remineralization. Toothmin toothpaste (Calcium Sucrose Phosphate) 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. It also actively neutralizes plaque acids – thus may be an effective option in the management of white spot lesions. These topical agents show promise in their ability to prevent enamel demineralization during orthodontic treatment, however clinical data comparing the efficacy of these remineralizing is lacking. Therefore, the purpose of this in – vivo study was to investigate the efficacy of remineralizing agents available commercially which claim to reduce demineralization when applied topically adjacent to orthodontic brackets.

**Aims objectives**

**The aims and objectives of the study were**

- To identify the association of demineralisation with orthodontic brackets on the tooth surface.
- To assess the effectiveness of GC Tooth Mousse or Toothmin toothpaste in prevention of demineralisation.
- To assess which of the either GC Tooth Mousse or Toothmin toothpaste provides better remineralisation.

## MATERIALS AND METHODS

The clinical scanning electron microscope study was conducted in the Department of Orthodontics and Dentofacial Orthopaedics, Seema Dental College and Hospital, Rishikesh, Uttarakhand and Indian Institute of Technology, Roorkee, Uttar Pradesh. The total study sample comprised of sixty sound premolar teeth from thirty subjects that will undergo fixed orthodontic mechanotherapy involving extraction.

**The test sample is divided into the following Groups**

- **Group 1:** The Group comprised of 15 subjects undergoing fixed orthodontic mechanotherapy and indicated for premolar extraction. The Group 1 is further subdivided into two groups:-
- **Group 1a:** GC Tooth Mousse application on the right side.
- **Group 1b:** Control Group as no application on left side
- **Group 2:** The Group comprised of 15 subjects undergoing fixed orthodontic mechanotherapy and indicated for premolar extraction. The Group 1 is further subdivided into two groups:-
- **Group 2a:** Tooth Min Toothpaste application on the right side.
- **Group 2b:** Control Group as no application on left side.

The study includes comparison of individual right side and left side Groups (Control Group) and overall comparison of statistics with each other (Group 1 and Group 2)

**Inclusion criteria includes**

- Subjects that have to undergo fixed orthodontic mechanotherapy and are indicated for extraction of maxillary first or second premolars.
- The teeth should be free from prior white spot lesions .
- Intact buccal enamel tooth surface.
- The teeth should be free from any enamel defects
- Patients who have not undergone any previous topical fluoride applications.
- Exclusion criteria include:
- Tooth that have any type of enamel loss
- Carious teeth
- Hypoplastic and Flourosed enamel
- Teeth with caries, restorations, attrition, cracks or fractures
- Non-compliant patients

**Preparation of sample**

**Method for bonding**

The selected subjects underwent oral prophylaxis with an ultrasonic scaler. After which 37% phosphoric acid gel was applied to the buccal surface of each tooth for 30 seconds. The teeth were then rinsed with a water spray for 30 seconds and dried with an oil free air source for 20 seconds, till the buccal surface of the etched teeth had a frosty white appearance. After surface preparation, the liquid primer (Transbond XT, 3M) was applied to the bracket base and etched surface , then cured for 10 seconds. All the brackets were bonded to the teeth using an adhesive (Transbond XT, 3M).

The adhesive was applied to the bracket base. Then the bracket was placed over the buccal surface of the tooth using a bracket holder. To ensure uniform thickness of the adhesive the bracket was compressed. Any excess adhesive was carefully removed using a small explorer without disturbing the bracket placement. Primer was applied around the edges of the bracket followed by the bonding adhesive being light cured for 10 sec. on each side of the bracket using a light emitting diode (L.E.D) curing unit – SDI Radii Plus which emits light of 430 to 480 nm at an intensity of 1000 $mW/cm^2$. The bonding procedure was carried out by single operator.

**Method of fabrication of custom tray**

An impression is taken with Alginate after completion of bonding. The impression is then poured using plaster of paris. A custom tray is fabricated using 1 mm thickness vacuum thermoformed sheet with the help of vacuum forming machine (Fig. 1).

**Method of application**

A custom tray was fabricated and given to the patient for application of GC Tooth Mousse or Tooth Min Toothpaste. An identification mark is made on the tray for indicating the site of application on the premolar surface (Fig. 2). The tray is rinsed before use by thoroughly under running water. A small amount (0.25gm) is extruded onto the tray at the indicated mark and applied to the tooth. The tray is then left undisturbed for 15 minutes.


**Fig. 1. Vacuum forming machine**

After which the tray is to be removed and any residual amount is to be expectorated. Any residual GC Tooth mousse / Tooth Min toothpaste in the tray should be rinsed or brushed off under running water immediately after use. Subjects of both Groups 1 and 2 were explained about the method of application and were instructed to apply the agent daily once at night after brushing their teeth for a period of 90 days. The method of application was demonstrated to the patient on day 1. Both groups were recalled on 21 days follow up. The oral hygiene instructions were reinforced during each appointment.

**Fig. 2. Custom tray with mark indicating site of application**

## Method of preparation of sample

The teeth are extracted after 90 days of application of the topical agents for both groups. All extracted teeth were cleaned thoroughly and stored using artificial saliva in plastic containers, which were then labelled. The sample is prepared for investigation via Scanning Electron Microscope. The buccal cusps of all teeth were cut off using carbide cutting disk. After that all tooth samples, the sectioned buccal cusps were coated with gold-palladium (Fig. 3, Fig. 4) in sputter coater (BAL-TEC SCD 005) (Fig ) to generate the secondary electrons necessary for image formation. Coated samples were scanned under electron microscope (LEO 435VF) (Fig. 6, Fig. 7) to assess the enamel surface. Surface images of the sample for both groups were scanned at X1000.

**Fig. 3. Sectioned teeth placed in in sputter coater (BAL-TEC SCD 005)**

**Fig. 4. section teeth sample after gold-pallidium sputter coating**

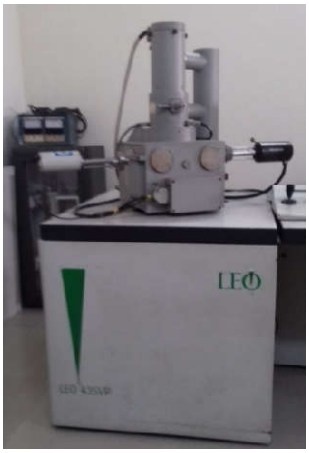




**Fig. 5. Sputter coater (BAL-TEC SCD 005 )**

**Fig. 6. Teeth sample placed in SEM (LEO 435VF)**

Statistical procedures were carried out in 2 steps

- Data compilation and presentation
- Statistical analysis

Statistical analysis was done using Statistical Package of Social Science (SPSS Version 20; Chicago Inc., USA). Data comparison was done by applying student t-test, chi square test and likelihood ratioto find out the statistical significance of the comparison Quantitative variables were compared using mean values and qualitative variables using proportions. To assess intra-observer error all the SEM images were scored by the same observer three times at the time interval of 7 days to eliminate the method errors calculated, as recommended by Intraclass Correlation Test. When intraobserver and interobserver correlation was calculated it was found that all the coefficient of reliability values were more than 0.7 which is indicative of highly reproducible values as shown in Tables 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, Table 8.

**Table 1. Intra class correlation coefficient to assess the inter observer reliability for measuring enamel roughness among group 1 subgroup a (cases)**

| | Intra-class Correlation | 95% Confidence Interval | | Significance |
|---|---|---|---|---|
| | | Lower Bound | Upper Bound | |
| Single Measures | .861 | .707 | .946 | .000 |
| Average Measures | .949 | .879 | .981 | .000 |

**Table 2. Intra class correlation coefficient to assess the inter observer reliability for measuring enamel roughness among group 1 subgroup b (controls)**

| | Intra class Correlation | 95% Confidence Interval | | |
|---|---|---|---|---|
| | | Lower Bound | Upper Bound | Significance |
| Single Measures | .957 | .902 | .984 | .000 |
| Average Measures | .985 | .965 | .995 | .000 |

**Table 3. Intra class correlation coefficient to assess the inter observer reliability for measuring enamel roughness among group 2 subgroup a (cases)**

| | Intra class Correlation | 95% Confidence Interval | | |
|---|---|---|---|---|
| | | Lower Bound | Upper Bound | Significance |
| Single Measures | .925 | .833 | .972 | .000 |
| Average Measures | .974 | .938 | .990 | .000 |

**Table 4. Intra class correlation coefficient to assess the inter observer reliability for measuring enamel roughness among group 2 subgroup b (controls)**

| | Intra class Correlation | 95% Confidence Interval | | |
|---|---|---|---|---|
| | | Lower Bound | Upper Bound | Significance |
| Single Measures | .924 | .831 | .971 | .000 |
| Average Measures | .973 | .937 | .990 | .000 |

### Method of evaluation

All the images were scored by single examiner. All the SEM pictures were scored from 0 to 5, based on comparative surface roughness.

The scores were graded as follows

- 0 - 1 mild roughness
- 2 - 3 moderate roughness
- 4 - 5 severe roughness

The data was then compiled systematically. A master table was prepared and the total data was subdivided and distributed meaningfully and presented as individual tables along with graphs.

## RESULTS

The clinical scanning electron microscope study was conducted in the Department of Orthodontics and Dentofacial Orthopaedics, Seema Dental College and Hospital, Rishikesh, Uttarakhand and Indian Institute of Technology, Roorkee, Uttarakhand. The total study sample comprised of sixty sound premolar teeth from thirty subjects that will undergo fixed orthodontic mechanotherapy involving extraction. Data collected by scoring of the SEM images was sent for statistical evaluation and the results obtained are discussed below – Group 1 – As shown in Table no. 9, Graph no. 1 mean value for subgroup a is 1.680 ± 0.8445 and subgroup b (control) is 4.040 ± 0.8517.

**Table 5. Inter observer reliability for Group 1 subgroup a**

| | Intra class Correlation | 95% Confidence Interval | | |
|---|---|---|---|---|
| | | Lower Bound | Upper Bound | Significance |
| Single Measures | .996 | .987 | .999 | .000 |
| Average Measures | .998 | .993 | .999 | .000 |

**Table 6. Inter observer reliability for Group 1 subgroup b**

| | Intra class Correlation | 95% Confidence Interval | | |
|---|---|---|---|---|
| | | Lower Bound | Upper Bound | Significance |
| Single Measures | .986 | .959 | .995 | .000 |
| Average Measures | .993 | .979 | .998 | .000 |

**Table 7. Inter observer reliability for Group 2 subgroup a**

| | Intra class Correlation | 95% Confidence Interval | | |
|---|---|---|---|---|
| | | Lower Bound | Upper Bound | Significance |
| Single Measures | 1.000 | 1.000 | 1.000 | .000 |
| Average Measures | 1.000 | 1.000 | 1.000 | .000 |

**Table 8. Inter observer reliability for Group 2 subgroup b**

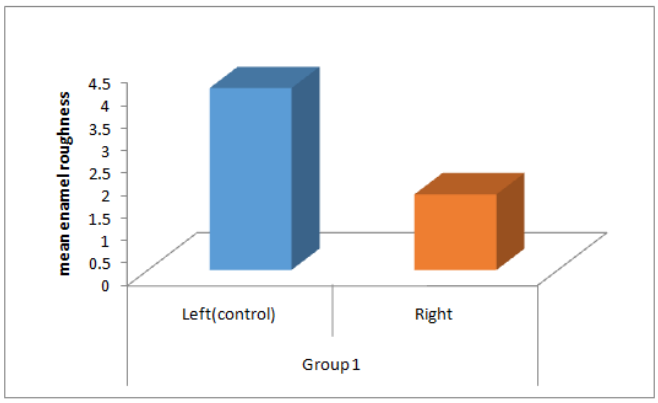
| | Intra class Correlation | 95% Confidence Interval | | |
|---|---|---|---|---|
| | | Lower Bound | Upper Bound | Significance |
| Single Measures | .970 | .914 | .990 | .000 |
| Average Measures | .985 | .955 | .995 | .000 |

**Table 9. Descriptive and Comparative assessment of enamel roughness scores for group 1**

| Group | Side | Mean | Std. Deviation | t value | p value |
|---|---|---|---|---|---|
| Group 1 | Left(control) | 4.040 | 0.8517 | 7.621 | <0.01* |
| | Right | 1.680 | 0.8445 | | |

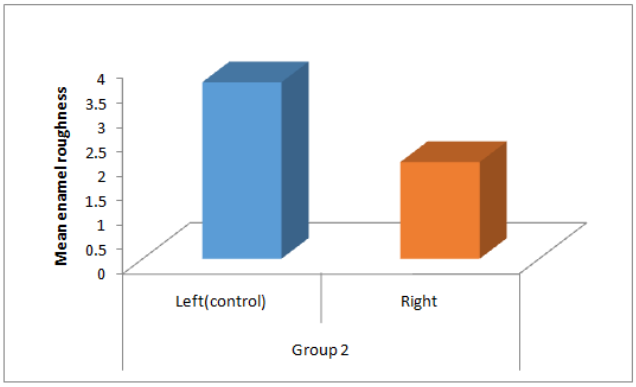
**Table 10. Descriptive and Comparative assessment of enamel roughness scores for group 2**

| Group | Side | Mean | Std. Deviation | t value | p value |
|---|---|---|---|---|---|
| Group 2 | Left(control) | 3.613 | 0.9372 | 4.912 | <0.01* |
| | Right | 1.980 | 0.8833 | | |




**Graph 1. Descriptive and Comparative assessment of enamel roughness scores for group 1**

When subgroup a was compared with subgroup b a high statistically significant (P <0.01) relation was found. The mean for subgroup a is lesser than that for subgroup b indicating a difference and significant amount of decrease in surface roughness of enamel as compared to subgroup b or control. Group 2 – As shown in Table no. 10, Graph no. 2 mean value for subgroup a is 1.980± 0.8833 and subgroup b (control) is 3.613± 0.9372. When subgroup a was compared with subgroup b a high statistically significant (P <0.01) relation was found. The mean for subgroup a is lesser than that for subgroup b indicating a difference and significant amount of decrease in surface roughness of enamel as compared to subgroup b or control. Thus a statistically significant amount of remineralisation has taken place in both Group1 and Group 2, in accordance with the comparison of results of subgroup a and subgroup b in both groups respectively. The overall comparison of Group 1 and Group 2 is shown in Table no.11 and Graph no. 3.




**Graph 2. Descriptive and Comparative assessment of enamel roughness scores for group 2**

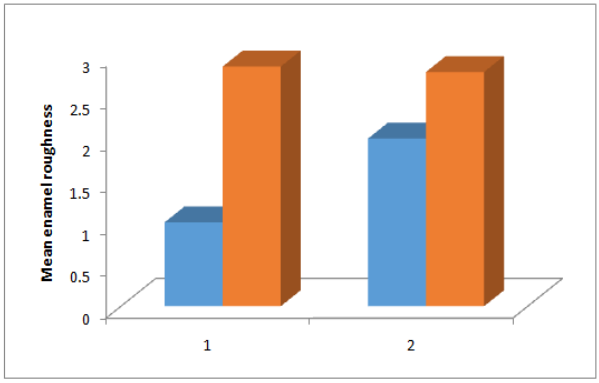
The overall mean for Group 1 is 2.86 ±1.461 and that for Group 2 is 2.796 ± 1.220. Although is a minute difference in the mean of Group 1 and Group 2 indicating slightly more

surface roughness in Group 1 ,the result is statistically insignificant with the P value of 0.856. Table no.12 and Graph no. 4 depicts the comparative assessment of sub group a in both Group 1 and Group 2.

**Table 11. Overall Comparative assessment of enamel roughness scores for group 1 & 2**

| Group | Mean | Std. Deviation | t value | p value |
|---|---|---|---|---|
| 1 | 2.86 | 1.461 | .182 | 0.856 |
| 2 | 2.796 | 1.220 | | |

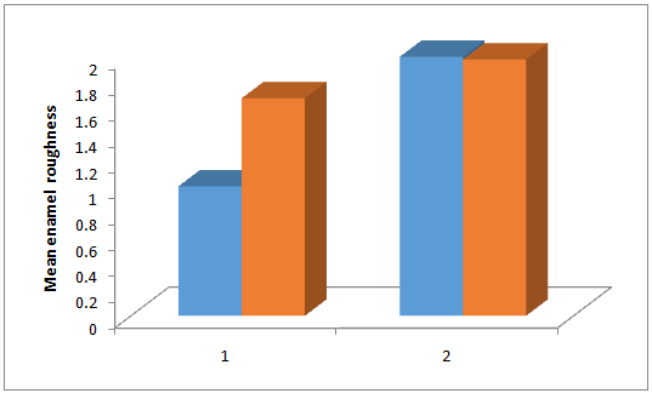
The mean for Group 1 subgroup a is 1.68 ± 0.844 and the mean for Group 2 subgroup a is 1.98 ± 0.883 indicating a difference and slightly more surface roughness in Group 2




**Graph 3. Overall Comparative assessment of enamel roughness scores for group 1 & 2**

**Table 12. Comparative assessment of enamel roughness scores for sub group a (right side) for group 1 & 2**

| Group | Mean | Std. Deviation | t value | p value |
|---|---|---|---|---|
| 1 | 1.68 | 0.844 | -.951 | 0.350 |
| 2 | 1.98 | 0.883 | | |




**Graph 4 Comparative assessment of enamel roughness scores for sub group a (right side) for group 1 & 2**

**Table 13. Chi square tests for scores of surface roughness**

| Chi-Square Tests | | | |
|---|---|---|---|
| | Value | df | Asymp. Sig. (2-sided) |
| Pearson Chi-Square | 7.750[a] | 12 | 0.804 |
| Likelihood Ratio | 9.107 | 12 | 0.694 |
| Linear-by-Linear Association | 0.034 | 1 | 0.854 |
| N of Valid Cases | 60 | | |

Subgroup a. The result however is statistically insignificant with a P value of 0.350. On evaluation with Pearson's chi square and likelihood ratio test as seen in Table no. 13, the results were insignificant with P value > 0.05, which indicates increased roughness in controls compared to cases. NOTE- Intra class correlation coefficient value more than 0.7 is acceptable to show that all the raters have good reliability in recording the enamel roughness. On evaluation with Pearson's chi square and likelihood ratio test, the results were insignificant with p value>0.05, which indicates increased roughness in controls compared to cases.

## Conclusion

Demineralization of enamel surrounding orthodontic brackets is a significant clinical problem during and after orthodontic treatment. It is a major element of risk to patients when considering the risk benefit balance of orthodontic treatment. Many techniques have been proposed in literature for the correction of the unesthetic appearance of tooth due to the white spot lesions. An effort has been made to determine and compare the efficacy of GC Tooth Mousse and Toothmin toothpaste with regard to protection of the enamel around the bracket from demineralization during orthodontic treatment. The results have clinical implications for the establishment of the best product available commercially topical application that would help in prevention of white spot lesions – thus help to overcome the undesired effect of the Orthodontic treatment.

## The following conclusions were drawn

- Topical application of the remineralizing agents – GC Tooth Mousse and Toothmin toothpaste was effective in preventing enamel demineralization.
- The ability of Toothmin toothpaste in preventing demineralization was similar to that of GC Tooth Mousse.
- It was observed that the topical application of GC Tooth Mousse, Group 1 subgroup a and Toothmin toothpaste, Group 2 subgroup a was associated with a significantly statistical variation in the reduction of mean surface roughness when compared with the control Group 1 subgroup b , Group 2 subgroup b samples indicating that both GC Tooth Mousse and Toothmin toothpaste can be used by the orthodontic patients as a remineralising agent for the prevention of development of white spot lesions.

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

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