A Comparative Study on The Synergistic Effect of Different Remineralizing Topical Agents And Biofilm Modifiers on Enamel Demineralization Around Orthodontic Brackets: An In Vivo Study

Mahmoud B Mohamed¹, Saleh A Saleh¹, Wesam El-din R Gouda¹, Mohamed A Abbas²

ABSTRACT

Aim: To evaluate and compare the remineralizing potential of the two remineralizing topical agents (TCP, MI PAST PLUS) with and without xylitol. Subjects and Methods: This study was conducted on twenty patients five in each group. Group1. The teeth were treated by clinpro white varnish. Group2. The teeth were treated by MI Paste Plus™, Group3. The teeth were treated by clinpro white varnish with xylitol, and Group 4 where the teeth were treated by MI past plus with xylitol. The remineralizing effect was evaluated through photographic analysis of color changes using USB digital microscope at 0, 3, 6 and 9 months (T0, T1, T2 & T3). In addition, Micro hardness test and energy dispersive x-ray analysis were carried out on premolars obtained from patients indicating for therapeutic extraction for orthodontic treatment. Results: The present study revealed that: (1) For Vickers surface hardness. The highest mean values were recorded for Group 3. (2) For energy dispersive x-ray, the highest total enamel mineral profile contents were recorded for Group (3) For Color Change (ΔE) Assessment, at T1 as well as T2 the highest color changes were recorded for Group 2 and While at T3 the highest color changes were recorded for Group 1 Conclusion: The tooth pastes containing TCP has good effect on remineralization potential of the enamel in teeth and the addition of xylitol can enhance the potential effect of remineralizing topical agents.

INTRODUCTION

Enamel demineralization is one of the most undesired side effects of fixed orthodontic treatment (1,2). The brackets, bands, and wires and other attachments interfere in effective oral hygiene procedure, causing plaque gathering, which exacerbates the risk of demineralization (3). Studies have shown that white spot lesions (WSLs) can develop within 1 month after bonding (4). The prevalence of WSLs after orthodontic treatment varies from 2% to 96% (5).

Enamel demineralization not only violates the esthetic principle of orthodontic treatment but also damages tooth health. Besides all the
active preventative methods, early and accurate detection of WSLs and effective remineralizing treatments are also crucial. Sudjalim et al carried out a laboratory study to investigate the effect of sodium fluoride and 10% CPPACP on enamel demineralization adjacent to orthodontic brackets, and found that the use of both agents should be recommended for all orthodontic patients to provide preventive actions and potentially remineralize early enamel demineralization (6).

Previous studies indicated reduced demineralization around orthodontic brackets in vitro, and visual regression of WSLs following topical application of agents containing CPP–ACP complexes in vivo. It is believed that the combined application of CPP-ACP with fluoride provides a synergistic effect on enamel remineralization. Singh et al. (2016) study revealed that brushing twice daily with 1000 ppm of fluoride toothpaste along with 5 % NaF varnish or CPP-ACP plus crème was more effective in remineralizing the WSLs.

Several studied evaluated the ability of calcium phosphate and fluoride containing varnishes to inhibit enamel demineralization. Chunhua et al evaluated the remineralization effects of casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) paste on enamel lesions by assessing ultra-structure, nano mechanical properties, and compound and elemental analysis. Bröchner et al investigate the effect of topical applications of 10% casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) on white spot lesions (WSL) detected after treatment with fixed orthodontic appliances. The study demonstrated that topical treatment of white spot lesions after deboning of orthodontic appliances with a casein phosphopeptide-stabilized amorphous calcium phosphate agent resulted in significantly reduced fluorescence and a reduced area of the lesions after 4 weeks “natural” regression following daily use of fluoride tooth paste.

PATIENTS AND METHODS

This study was carried on 20 patients that were selected from the outpatient clinic of faculty of dental medicine Al-Azhar university Assuit branch and they were selected on the basis of:

1. Patient were in need for orthodontic treatment.
2. No previous trauma or orthodontic treatment
3. All permanent teeth erupted except third molar.
4. Patient need for extraction of first premolar teeth.
5. Good oral hygiene.
6. No esthetic restorations or decalcifications in the upper incisors and canines.
7. Age range between 14 and 20years.
8. No smoking habit.

Patient groups

The patients in the present study were randomly divided into 4 groups according to the type of remineralizing material into the following:

**Group 1**: Teeth treated by clinpro white varnish TRI CALCIUMPHOSPHATE(TCP). Calcium phosphate compound is functionalized tricalcium phosphate (f TCP), which is produced by the solid-state ball milling of beta-tricalcium phosphate and sodium lauryl sulfate.

**Group 2**: Teeth treated by MI Paste Plus™ casein phosphopeptides amorphous calcium phosphate group. Nanocomplex (Recaldent TM (CPP-ACP)) is a technology based in ACP stabilized by Casein phosphopeptides (CPP).

**Group 3**: Teeth treated by clinpro white varnish (TCP) with xylitol tooth paste. Xylitol, a five-carbon natural sugar alcohol, has been shown in many studies to be a successful dental caries–preventive natural carbohydrate sweetener after a total or partial substitution of dietary sucrose by this pentitol.
**Group 4:** Teeth treated by MI past plus with xylitol tooth paste. Polyols are sweeteners that are weakly metabolized (sorbitol) or not metabolized (xylitol) by cariogenic bacteria. Evidence supports that xylitol is noncariogenic, exhibits a dose and frequency dependent effect on dental plaque and mutans streptococci, and is safe.

After the brackets were bonded to the facial surface of all teeth. The lingual surface of first premolar teeth were etched and the button were bonded to the lingual surface of first premolar teeth of each group and used as a control side without any material application for energy dispersive x-ray analysis (EDEX) and micro hardness test after extraction.

**After the brackets were bonded** each patient give instruction to use the remineralizing materials around the brackets according to each group as the following: In (group 1): The patients instruct to use the (TCP) clinpro white varnish with 5% sodium fluoride (Clinpro, 3M ESPE Dental, USA) varnish three time daily by painting it on the labial surface of the anterior teeth around the brackets by brush used for this purpose for 4-7 minutes for effective use and not to eat or drink within 1 hour later. Repeated this action 3 times daily for 3 months, with follow up visit weekly. In (group 2): The group (CPP-ACP) subjects were advised to use pea size CPP-ACP plus crème (GC Tooth Mousse, Asia Pty. Ltd, Japan) on the facial tooth surfaces using a clean finger three times daily to keep the CPP-ACP plus crème over the teeth surfaces for at least 3-7 minutes following brushing their teeth with xylitol (Kodomo™, Lion, Japan) tooth paste for enhancement the remineralizing effect of MI past plus and not eat or drink for 1 hour later. Repeated this action 3 times daily for 12 weeks, with weekly follow up visit.

**Postoperative Evaluation**

**Color change** for all patient’s series of intra oral photographs from the facial surface of maxillary anterior and premolar teeth including (central, lateral, canine and first premolar teeth) were taken, representing base line reading (T0) before application of remineralizing materials. The color measurement of each specimen (T0) was performed using computational technique with a combination of a digital microscope, image processing software

**Energy Dispersive X-Ray Spectrometric Analysis (Edax).**

The sample of each subgroup were analyzed using energy dispersive X-ray analysis to determine the calcium and phosphorus mineral contents and the change in the level of these elements percentage between the buccal surface (treatment surface) and lingual surface (non treatment surface) which consider as control side, and the change in the level of the calcium and phosphorus mineral content between each group.

**Vicker Microhardness Test.**

The enamel surface hardness of the specimens was measured using digital vicker microhardness tester using 100g. Load/15 second from the buccal and lingual surface of each premolar tooth, where the lingual surface considers as control side for the buccal surface of each tooth. Demineralization of the enamel surface was evaluated quantitatively by cross-sectional microhardness testing (superficial microhardness analysis): indentations were made at the edge of the bracket base (0 µm) and at 100 and 200 µm distant from it. In all of these positions,
Statistical analysis:
All data were collected and tabulated for statistical analysis. Data were presented as mean and standard deviation values repeated measures analysis of variance (ANOVA) by SPSS 2019 that was used to determine the effect of different Remineralizing topical agents on tooth surface. One way ANOVA test was used to compare between the four different groups where a value of P < 0.005 was considered statistically significant.

RESULTS

1. Color Change (ΔE) Assessment

a. After three months
The highest color changes were recorded for Gr_2; (5.96±1.3∆E) followed by Gr_1; (5.4±1.1∆E) with intermediate color change for Gr_4; (3.88±0.6∆E) meanwhile the lowest color changes were for Gr_3; (3.12±0.5∆E). The difference in color changes for enamel between different experimental groups was statistically significant (F=9.7, P=0.0007<0.05) as shown in the table 1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Evaluation time</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr_1</td>
<td>5.4±1.1</td>
<td></td>
</tr>
<tr>
<td>Gr_2</td>
<td>5.96±1.3</td>
<td>0.0007*</td>
</tr>
<tr>
<td>Gr_3</td>
<td>3.12±0.5</td>
<td></td>
</tr>
<tr>
<td>Gr_4</td>
<td>3.88±0.6</td>
<td></td>
</tr>
</tbody>
</table>

Different letter in same column indicating statistically significant difference (p < 0.05). ns; non-significant (P>0.05)
*; significant (P<0.05)

b. After six months
The highest color changes were recorded for Gr_2; (5.98±1.1∆E) followed by Gr_1; (5.76±1.03∆E) with intermediate color change for Gr_4; (34.02±1.7∆E) meanwhile the lowest color changes were for Gr_3; (3.58±0.7∆E). The difference in color changes for enamel between different experimental groups was statistically significant (F=5.3, P=0.0101<0.05) as shown in the table.

c. After nine months
The highest color changes were recorded for Gr_1; (7.19±0.61 ∆E) followed by Gr_2; (6.35±0.35 ∆E) with intermediate color change for Gr_4; (3.92±0.47 ∆E) meanwhile the lowest color changes were for Gr_3; (3.42±0.14 ∆E). The difference in color changes for enamel between different experimental groups was statistically significant (F=75.3, P=<0.0001<0.05) as shown in the table.

2. Vickers surface hardness (HV)

a) At baseline stage; there was no-significant difference between enamel Vickers surface hardness for all experimental groups as indicated by one way ANOVA (F=2.7, P=0.0794 >0.05)

b) After re-mineralized stage, the highest mean ± SD values of Vickers hardness were recorded for Gr_3 (285.78±8.65 HV) followed by Gr_4 mean ± SD values (284.52 ±12.02 HV) then Gr_1 mean ± SD values (283.38 ±13.28 HV) meanwhile the lowest mean ± SD value was recorded for Gr_2 (259.815±23.82HV). The difference between enamel Vickers surface hardness for all experimental groups was statistically no-significant as indicated by one way ANOVA (F=0.1, P=0.9801>0.05), (Table 2).

3. Mineral profile change results by EDAX
The difference in total enamel mineral profile contents between baseline and all experimental groups was statistically significant as indicated by one way ANOVA (F=10.1, P=0.0001<0.05) as shown in the table 3.
Table (2): Vickers surface hardness (Mean ±SD) values for enamel (HV) at baseline and post-treatment with different experimental groups

<table>
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<th>Variables</th>
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<th>ANOVA</th>
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<tr>
<td></td>
<td>Re-mineralized</td>
<td>P value</td>
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<tr>
<td>Gr_1</td>
<td>283.38±13.28</td>
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</tr>
<tr>
<td>Gr_2</td>
<td>283.01±11.17</td>
<td>0.9801 ns</td>
</tr>
<tr>
<td>Gr_3</td>
<td>285.78±8.65</td>
<td></td>
</tr>
<tr>
<td>Gr_4</td>
<td>284.52±12.02</td>
<td></td>
</tr>
</tbody>
</table>

Different letter in same column indicating statistically significant difference (p < 0.05).
ns; non-significant (P>0.05)
*; significant (P<0.05)

Table (3): Mineral profile change results (%) in baseline and for all experimental groups after 3 months evaluation time

<table>
<thead>
<tr>
<th>Variable</th>
<th>Stage</th>
<th>Ca</th>
<th>P</th>
<th>Ratio</th>
<th>Total</th>
<th>Change%</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>27.9</td>
<td>16.7</td>
<td>1.670658683</td>
<td>22.3±1.07</td>
<td></td>
</tr>
<tr>
<td>Experimental Group</td>
<td>Gr_1</td>
<td>30.4</td>
<td>17.7</td>
<td>1.717514124</td>
<td>24.05±0.37</td>
<td>7.276507</td>
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<tr>
<td></td>
<td>Gr_2</td>
<td>28.9</td>
<td>17.1</td>
<td>1.69005848</td>
<td>23.8±0.34</td>
<td>3.043478</td>
</tr>
<tr>
<td></td>
<td>Gr_3</td>
<td>32.4</td>
<td>17.39</td>
<td>1.86319735</td>
<td>24.9±0.97</td>
<td>10.42378</td>
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<tr>
<td></td>
<td>Gr_4</td>
<td>31.8</td>
<td>17.2</td>
<td>1.84837209</td>
<td>24.5±0.73</td>
<td>8.979592</td>
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Statistics

<table>
<thead>
<tr>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001*</td>
</tr>
</tbody>
</table>

Different letter indicating significant (p<0.05) *; significant (p<0.05) ns; non-significant (p>0.05)

DISCUSSION

In the normal oral environment, the tooth structure undergoes continuous demineralization and remineralization. During orthodontic treatment, demineralization is more common and the tooth enamel is at a higher risk for caries which is due to food stagnation caused by the appliances and inadequate oral hygiene causing white spot lesions (WSLs). There are many possibilities to arrest or reverse the progression of the White spot lesions (13).

Efficient remineralization requires the exposure of enamel to low concentrations of calcium and phosphate ions for long periods of time. Thus, extraoral sources of calcium, phosphate and fluoride can change the cariogenic potential of dental biofilm (14).

In particular the action of efficient remineralization requires the exposure of enamel to low concentrations of calcium and phosphate ions for long periods of time. Thus, extraoral sources of calcium, phosphate and fluoride can change the cariogenic potential of dental biofilm (14).

The clinical use of calcium and phosphate ions for remineralization has not been successful in the
past, due to low solubility of calcium phosphates particularly in the presence of fluoride ions. For every 2 fluoride ions, 10 calcium ions and 6 phosphate ions are required to form one unit cell of fluorapatite (Ca$_{10}$(PO$_4$)$_6$ F$_2$)\textsuperscript{(15)}.

Many laboratory and clinical studies have demonstrated that the combination of fluoride and functionalized $\beta$-tricalcium phosphate ($\beta$TCP) produces stronger, more acid-resistant mineral relative to fluoride, native $\beta$-TCP, or $\beta$TCP alone. In contrast to other calcium-based approaches that seem to rely on high levels of calcium and phosphate to drive remineralization, $\beta$TCP is a low dose system designed to fit within existing topical fluoride preparations. The functionalization of $\beta$-TCP with organic and/or inorganic molecules provides a barrier that prevents premature fluoride-calcium interactions and aids in mineralization when applied via common preparations and procedures \textsuperscript{(16)}.

And in this study the methods of evaluation involved photographic analysis using USB digital microscope. A series of intra oral photographs from the facial surface of maxillary anterior and premolar teeth including (central, lateral, canine and first premolar teeth) were taken, representing base line reading (T0) before application of remineralizing materials and after three month, six months and nine months which represent (T1, T2 and T3). The evaluation after nine months to assist in long period of follow up.

The results of this study revealed that after three months of color evaluation (group 3) showed better clinical and experimental results followed by (group 4) then (group 1) and lastly (group 2). It was confirmed by the color change assessment test, where the lowest color change was in (group 3) and the highest color change was in (group 2).

The difference in color changes for enamel between different experimental groups was statistically significant where (P=0.0007<0.05).

Based on time effect the highest color change in group 1, 2 was after nine months and after six months in both group 3 and group 4. and lowest color change was recorded after three months for all groups. confirming that the xylitol aids in decreasing the demineralization and increased the remineralizing effect.

All groups showed their best results in the first three then its final effect was confirmed after 9 months in group 1, 2 and six months in group 3, 4 so the long follow up period is recommended for the optimal evaluation of the remineralizing effect of the tested material.

Experimentally after three months the xylitol addition has an increasing effect on the Ca and P total mineral content when added to both MI paste PLUS and TCP on the labial surface of teeth, that was confirmed by the EDAX total mineral content test where Group 3(24.9A±0.97at. %) showed the highest records followed by group 4 (24.5A±0.73at. %) with intermediate mean for Gr_1 and 2; (24.05A±0.37at. % and 23AB±0.34at. % respectively) while the lingual surface showed the lowest mineral content.

Experimentally after three months the application of remineralizing material the highest mean ± SD values of Vickers hardness were recorded for Gr_3 (285.78±8.65HV) followed by Gr_4 mean ± SD values (284.52±12.02HV) then Gr_1 mean ± SD values (283.38±13.28HV) meanwhile the lowest mean ± SD value was recorded for Gr_2 (259.815±23.82HV).

Totally it was found that TCP treated group with xylitol recorded statistically significant (p=0.0006<0.05) higher hardness test, lowest color change and highest mineral contents than CPP-ACP.
with xylitol treated group, followed by TCP group and lastly MI Paste PLUS group.

This result was in disagreement with Abufarwa et al. (2019) a study which reported that CPP-ACP + fluoride have led to better remineralization capacity the limitation of this study included period of remineralization used in the study which could not remineralize artificial caries completely. 16

This result was also in a disagreement with Balakrishnan et al. evaluated the remineralizing potential of three different remineralizing agents (GC tooth Mousse, Clinpro tooth creme and SHY-NM) on demineralized tooth surfaces using micro-CT and microhardness. Forty-five freshly extracted mandibular premolars were collected and enamel specimens were prepared. The study concluded that CPP – ACP showed the better remineralizing potential than the other two agents and there was no statistically significant difference between f-TCP and CSP groups. 29

The results of this study were in agreement with Camila Nassur et al. evaluate the intra-.and inter-examiner reliability of two methods, namely, digital caliper [DC] and computerized image analysis software (Image Tool [IT] version 4.1) for measuring the area of an active enamel demineralization lesion. The intra-examiner correlation using the IT method showed excellent reproducibility.

Computerized image analysis with the Image Tool software demonstrated higher intra-.and inter-examiner reliability than the digital caliper method, in estimating the area of the enamel demineralization lesion 27.

CONCLUSION

From our results it could be concluded that

1. Remineralizing agents containing Calcium phosphate components are able to remineralize the early enamel carious lesion
2. TCP dentifrice appears to have a greater effect on remineralization of carious-like lesions.
3. The MI Paste Plus demonstrated a protective effect against demineralization and provide smoothed surface for the treated teeth.
4. The tooth pastes containing TCP enhance the remineralization potential of the enamel in teeth and more efficient Than CPP-ACP.
5. Addition of xylitol enhance the potential effect of remineralizing topical agents.
6. Patient under high caries risk like orthodontic patient, it is advisable to use the CPP-ACP and TCP with addition of xylitol for extended application of time in order to attain more benefits of remineralization regimens application in the form of tooth paste.

REFERENCE


15. Moufida Abufarwa, Texas A&M University College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246, 2019 by The EH Angle Education and Research Foundation, Inc.

A Comparative Study on The Synergistic Effect of Different Remineralizing Topical Agents And Biofilm Modifiers on Enamel Demineralization Around Orthodontic Brackets: An In Vivo Study

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Abstract:

The aim of this study was to evaluate the effectiveness of calcium fluoride and fluorapatite with and without the addition of zinc oxalate to remineralize cervical white spot lesions on teeth around orthodontic brackets. The study was conducted in two stages: an initial assessment of the mineral content of the teeth in the study using a digital microscope and after three months of orthodontic treatment, the cervical portion of the teeth was separated from the roots and immersed in a test solution with a period of four months. The enamel surface was not immersed. The orthodontic segments were bonded to the teeth of all patients, and the study continued to evaluate remineralizing materials during a nine-month period.

The first stage divided the samples into four groups according to the type of remineralizing materials used, while the second stage divided the samples into three groups according to the addition of zinc oxalate to calcium fluoride and fluorapatite. After three months, all teeth were measured and a standardized cycle was performed using a digital microscope to assess the color of all anterior and posterior teeth, and the initial measurement was repeated after nine more months.

The results showed that the combination of calcium fluoride and zinc oxalate led to the highest color test mean, followed by calcium fluoride, and then calcium fluoride and fluorapatite. There was no significant difference between the groups. The study also showed that there was no significant difference between the four groups after exposure to the digital microscope that measures the mineral content of the tooth through the examination of differences in color and the percentage of calcium and phosphorus.

The researchers concluded that, in the case of patients at high risk of erosion, it is recommended to use a combination of calcium fluoride and zinc oxalate for a long period to achieve more benefits from remineralization or to use calcium fluoride, which has a similar effect but without the addition of zinc oxalate.

Keywords: Calcium fluoride, topical remineralization, enamel, biofilm modifiers.