Evaluation of three remineralizing agents on surface roughness of enamel

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Background and Objective: This study aimed to assess and compare, in vitro, the efficacy of Casien phosphopeptide-Amorphous calcium phosphate fluoride (CPP-ACP F), Sodium fluoride (NaF) mouth rinse and orthokin tooth paste in treatment of white spot lesions (WSLs) by examining changes in surface roughness of the enamel teeth following pH cycling.

Methods: This in vitro experimental study was conducted on Forty (N=40) extracted sound maxillary premolars which were extracted for orthodontic purposes. Each tooth was soaked in demineralizing solution at 37^{o}C for14 days to produce a white spot lesion. Then samples were coded from 1 to 40 for identification purposes and middle of buccal surface were subjected to profilometric analysis for obtaining enamel surface roughness parameters prior to any treatment (1st roughness recording). Afterward, specimens were randomly divided into four groups of 10 teeth. Each group was immersed and kept in an artificial saliva solution. In group 1 Specimens were treated with ortho kin toothpaste, in group 2 samples were immersed in 10 ml of 0.05% NaF mouthwash, every specimen in group 3 (control group) was immersed in 10ml artificial saliva and in group 4 specimens were treated with MI Paste Plus. After completion of 14 days application of remineralizing agents, a new assessment of enamel surface roughness was obtained by using profilometeric analysis.

Results: The enamel surface roughness of all specimens was decreased after remineralization. There was a statistically significant difference between the roughness measured before and after the application of an experiment in GC group and ortho Kin group. The results showed that both Group 4 (GC, MI paste) and Group 2 (Mouth wash) group had a significant effect on the surface roughness when compared to other two groups. However, the remineralizing effect of Group 1 (Kin toothpaste) and control group were not significant.

Conclusions: Within the limitations of this in vitro study, the results showed that MI Paste Plus and NaF are effective for the treatment of WSLs. Although Kin toothpaste had an efficacy superior to artificial saliva, its remineralizing effect seems to be inconsistent.

Keywords: Surface roughness, White spot lesions, MI paste plus.

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Introduction

Despite extensive efforts made so far to prevent dental caries, dental caries is still considered as the most common chronic disease during childhood and adolescence and they are quite prevalent in some societies.[1] White spot lesions (WSL) or enamel decalcification is the clinical manifestation of early enamel caries and attributes to the prolonged retention and accumulation of dental bacterial plaque. The white appearance of these lesions is the result of different optical reflections due to mineral loss in the surface or subsurface enamel,[2,3] Orthodontic patients developed significantly more WSLs than non-orthodontic patients. According to

the literature, the prevalence of WSLs following orthodontic treatment is varied between 2 to 96% and they mainly happen on cervical and middle thirds of the buccal surface of first molars, premolars, lateral incisors and canine teeth.[4,5] It is difficult for patients to achieve proper oral hygiene in the presence of orthodontic appliances. In addition, due to the existence of bands, brackets, elastics, hooks, and springs, removing dental plaques is a challenging task,[6] leading to the formation of WSLs and caries in progressive stages, particularly in those with unacceptable oral hygiene at the pretreatment examination and during treatment.[7] Therefore, it seems essential to adopt some preventive and therapeutic measures before and during orthodontic treatment to lower the probability of developing incipient caries.

It is impor-tant for white spot lesions to be prevented, but fortunate-ly these demineralized areas can be remineralized easily because the outermost layer of enamel remains intact and the underneath layer can be remineralized by absorbing minerals.[8] Fluoridated toothpaste and mouthwashes contain fluoride are considered the most effective agents for preventing enamel demineralization.[9,10] Fluoride is a protective agent which can prevent or reverse dental caries via the following me-chanisms: 1) adsorption to minerals of the enamel and enhancing protective mechanisms against acid dissolu-tion 2) counteracting bacterial enzymes, which leads to bacterial plaque inactivity, 3) speeding up the remineralization process by attracting calcium ions in the partia-lly demineralized subsurface crystals in carious lesions.[11]

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) commercially available as GC Tooth Mousse has been used to reduce or eliminate the incidence of enamel demineralization. 'GC Tooth Mousse plus' is water-based, sugar-free cream containing the active ingredient CPP-ACP, when applied to tooth binds with biofilms, plaque, bacteria, hydroxyapatite and surrounding soft tissue localizing the bioavailable calcium and phosphate. The flavored Tooth Mousse stimulates the flow of saliva which enhances the effectiveness of CPP-ACP. *In vitro* and *in vivo* studies have confirmed the effectiveness of the product to decrease the incidence of enamel demineralization.[12,13] When adequate levels of calcium and phosphate ions are combined with fluoride ions (CPP-ACPF), it has been shown that this combination can result in substantial remineralization of enamel lesions. Fluoride combined with CPP-ACP is demonstrated to be incorporated into the body of the white spot lesion and is not localized at the outermost surface layer of enamel. The diffusion of fluoride ions together with calcium and phosphate ions deep into the lesion enables substantial crystal growth (remineralization) throughout the body of the lesion.[14,15]

This study aimed to assess and compare, in vitro, the efficacy of CPP-ACP F (MI paste plus), NaF mouth rinse (orthokin mouth wash) and orthokin toothpaste on enamel surface roughness when used as remineralizing agents for WSLs.

Methods

This in vitro experimental study was conducted on Forty (N=40) extracted sound maxillary premolars which were extracted for orthodontic purposes with no discoloration, caries, restorations, cracks, and/or fractures.

The teeth were stored in normal saline at room temperature till testing date. Afterward, the root part of each tooth was embedded in self-curing acrylic resin. The enamel surfaces were painted with an acid-resistant nail varnish, except for a window of 2 × 3 mmat the center of the buccal surface, to prevent contact with the demineralizing and remineralizing agents.

When the varnish dried, each tooth was soaked in demineralizing solution at 37°C for14 days to produce a white spot lesion. The demineralizing solution consisted of (3 mmol/L of monopotassium phosphate, 3 mmol/L of calcium chloride dihydrate, and 0.1 M of lactic acid 85% solution with the pH adjusted to 4.5 with about 5 g of potassium hydroxide)[16]. The solution was changed daily during this experiment. To ensure equal acid potency for all teeth, a fresh solution was used for each tooth.

Following completion of the demineralization process, the teeth were washed cautiously in distilled water. Consequently,

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each tooth displayed an artificial enamel lesion of known size (Figure 1).
Then samples were coded from 1 to 40 for identification purposes and middle of buccal surface was subjected to profilometric analysis (SJ-401 surface profilometer, Mitutoyo Corporation, Japan) for obtaining enamel surface roughness parameters before any treatment (1st roughness recording) (Figure 2).
Afterward, specimens were randomly divided into four groups of 10 teeth. Each group was immersed and kept in artificial saliva solution (2.200 g/L of gastric mucin, 0.381g/L of sodium chloride, 0.213 g/L of calcium chloride dihydrate, 0.738 g/L of dipotassium phosphate, and 1.114 g/L of potassium chloride;[16] pH was adjusted to 7 with 85% lactic acid at 37° C with 100% humidity for 14 days and subjected to the different treatment protocol (Figure 3). Table 1 illustrates the chemical composition of the remineralizing agents used in this study.
Group 1 Specimens were treated with ortho kin toothpaste every 12 hours for five minutes then paste was wiped by soft cotton without rinsing, followed by immersing specimens in a freshly pre pared artificial saliva solution (Figure 4).
Group 2 samples were immersed in 10 ml of 0.05% NaF mouthwash (ortho kin mouthwasth) for five minutes every 12 hours (twice a day), then without rinsing, samples were immersed in a fresh artificial saliva solution (Figure 5).
Group 3 (control group) Specimens were immersed in 10ml artificial saliva and no other treatment were applied And artificial saliva was refreshed twice a day (Figure 6).
Group 4 Specimens were treated with MI Paste Plus (GC America, Alsip, Ill) every 12 hours for five minutes then paste was wiped without rinsing, followed by immersing specimens in a freshly prepared artificial saliva solution (Figure 7).

Figure 1. Artificial white spot lesion created on tooth enamel.

Figure 2. Profilometer device

Figure 3. Samples on artificial saliva

Figure 4. Ortho kin tooth paste and the tooth paste on WSLs of the samples

Figure 5. Ortho kin mouth wash used in the study.

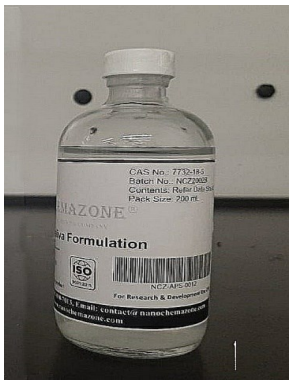

Figure 6. Artificial saliva used in the study.

The solution of artificial saliva in each container was changed after each treatment. After 14 days and visual verification of white spot lesion appearance on all sample teeth, all samples will be removed from artificial saliva. After completion of 14 days application of remineralizing agents, a new assessment of enamel surface roughness was obtained by using profilometeric analysis.

Figure 7. MI paste plus used in the study.

Data were entered and analyzed by the Statistical Package for Social Sciences (SPSS, version 22). Paired t-test was used to compare the means of enamel roughness before and after the treatment protocol. One-way Analysis of Variance (ANOVA) was used to compare the means of the differences between the reading (after – before) among the four study groups. A post hoc test (LSD) was used to compare each two means (after doing the ANOVA test). A '*P* value' of less than 0.05 was considered as statistically significant.

Results

Mean and measurement results of enamel surface roughness before and after treatment protocol are shown in Table 2.

Table 2 describes the differences in enamel surface roughness after immersion in the demineralization solution. The enamel surface roughness of all specimens was decreased after remineralization to a very similar extent.

There was a statistically significant difference between the means of enamel roughness measured before and after application of experiment in GC (MI paste) and ortho Kin (mouth wash) groups ($P < 0.05$) as shown in Table 2.

Analysis of covariance (ANOVA) showed that there was a significant difference in the effect of the remineralizing agents on the surface roughness between the groups. The Least Significant Difference (LSD) test showed that there was a significant difference in the effect on the surface roughness between the groups. The Least Significant Difference (LSD) test showed that there was a significant difference in the effect on the surface roughness between Control group and both Group 2 (Orthokin mouth wash) and Group 4 (MI paste plus) with no significant difference between Group 2 (Orthokin mouth wash) and Group 4 (MI paste plus).

There was no significant difference in the remineralizing effect between Group 1 (Kin toothpaste) and all other Grouops as seen in Table 3.

Table 1. Chemical composion of remineralizing agents

Remineralizing agents	Composition	Company
Orthokin toothpaste	Sodium fluoride (1450ppm), Cetylpyridinium chlode, Provitamin B5, Vitamin E acetate	Kin Laboratories, Spain
Orthokin mouth wash	Zinc acetate (0.34%), Chlorhexidine digluconate (0.06%), Sodium fluoride (0.22%)	Kin Laboratories, Spain
MI paste plus	Casien phosphopeptide-Amorphous calcium phosphate fluoride (CPP-ACP) , glyceril, propynele glycol, sodium fluoride, sodium saccharin	GC Corporation, Japan

Table 2. Means and t-test of enamel roughness (µm) before and after treatment protocol

Enamel roughness (µm)					
	Before		After		
Groups	**Mean**	**(±SD)**	**Mean**	**(±SD)**	**p**
Group 1	0.83	(+0.117)	0.72	(±0.136)	0.13
Group 2	0.89	(+0.053)	0.71	(±0.067)	0.001*
Group 3	0.61	(+0.092)	0.59	(±0.071)	0.65
Group 4	0.85	0.135	0.67	(±0.114)	0.01*

*Statistically significant $P < 0.05$

Table 3. Means of the differences (after and before) in the enamel roughness (µm) among the four study groups.

Groups	N	Mean difference	(±SD)	p (ANOVA)	LSD groups	p
Orthokin tooth paste	10	0.11	(+0.117)	0.001	G1 X G2	0.17
Orthokin mouth wash	10	0.18	(+0.053)		G1 X G3	0.18
Control	10	0.02	(+0.092)		G1XG4	0.32
MI paste plus	10	0.18	0.135		G2 X G3	0.003*
Total	40	0.49	(±0.143)		G2XG4	1
					G3XG4	.0004*

*Statistically significant $P < 0.05$

Discussion

The aim of this study was to evaluate the impact of three remineralizing different additives on surface roughness on white spot lesions when subjected to pH cycling. When the changes in surface roughness were evaluated using a profilometer, it was found that pH cycling induced an increased surface roughness across all groups.

The lack of significant difference in surface roughness of both orthokin tooth paste group and control group suggests that despite their protective action on the enamel and increase in surface mineralization, they are unable to cause or prevent changes in surface roughness induced in the enamel following pH cycling.

In a systematic review by Chen et al.[17] on the effect of remineralizing agents on WSLs after orthodontic treatment, they have suggested that NaF, CPP-ACP, and MI Paste Plus are effective materials for treatment of enamel lesions. However, no consensus has been reached about the most suitable and reliable technique for the treatment of WSLs. In the current study, a demineralizing solution with a pH of 4.5 was used to induce WSLs. The main composition of demineralizing agents often includes calcium, phosphorous and acetic acid or lactic acid, which is determined based on the desired pH and duration of exposure for the creation of lesions. Based on the previous studies, the pH of demineralizing solutions varied from 3.5 to 5, also, the duration of immersion in the solution ranged from two hours to 14 days.[18-20] In the present study, in order to create WSLs, the samples were immersed in a demineralizing solution with a pH of 4.5 for 14 days.

Different methods have been used to assess the degree of remineralization; for instance, Sudjalim et al.[4] used quantitative light-induced fluorescence. Mohan et al.[21] used scanning electron microscopy. DIAGNOdent[22] also has been used. Behroozibakhsh et al.[23] used more precise methods including micro-computed tomography. One of the most common and reliable methods for this purpose is the assessment of change in surface roughness using profilometer.[24] In the present study, surface roughness values were measured after demineralization and remineralization of specimens.

Statistical analysis showed after treatment, the maximum decrease in surface roughness occurred in group 2 and group 4 that samples were treated with NaF and MI Paste Plus respectively.

A treatment with CPP-ACP caused the greatest reduction in the enamel surface roughness when compared to the other two methods. CPP-ACP paste applied to the enamel surface in the oral cavity can bind 25 calcium ions, 15 phosphate ions, and 15 fluorine ions per molecule, and it can stabilize calcium phosphate in oral fluids. As a result, the pH of the oral cavity remains alkaline, which limits bacterial adhesion to the surface enamel, and it increases the process of remineralization and prevents the release of hydroxyapatite. The presence of phosphoryl residues may also sustain the amount of calcium phosphate ions in the oral cavity for a longer time than is possible with fluoride application. The reduction of the demineralization volume after application of MI paste plus was mainly due to an increase in the mineral content in the body of the lesion and a concomitant decrease in lesion depth especially after 14 days when compared to the control groups.[25]

Similar results were obtained by Shetty et al[26]; in their study, the application of MI Paste Plus was more effective than CPP-ACP and NaF for the treatment of enamel lesions following orthodontic treatment. Ballard et al.[5] compared three commercially available materials (MI Paste Plus, a toothpaste containing Novamin and 5000 Prevident) and they realized that none of these products had any superiority over the others. However, in our study, MI Paste Plus and NaF were significantly superior to the control group. The difference in the results of two studies may be due to the use of a different method of evaluation for determining the effect of remineralization materials.

The results of the present study indicate that the application of orthokin mouth wash may reduce WSLs, the application of orthokin mouth wash resulted in a significant decrease in surface roughness.

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If a high dose of fluoridated toothpaste is used locally, the arrested lesion will remain the same size and frequently becomes stained with organic debris. Moreover, natural remineralization produces greater resistance to further dissolution because components are replaced with less -soluble substances having larger crystals during remineralization. This phenomenon has been reported as plugging of the diffusion pathways of enamel by hydroxyapatite crystals and as hyper-remineralization.[27]

In the current study, results of ANOVA revealed a significant difference in surface roughness of groups treated with MI Paste Plus and NaF with the control group.

The remineralization process on demineralized enamel may occur when the pH of the saliva is in a neutral state (when the saliva's buffering ability is stable) and when there are enough Ca2+ and PO43- ions in the saliva. Factors that can help remineralization are saliva, mouthwash, topical fluoride, CPP-ACP, and toothpaste.[28] According to Nakashima, et al., toothpaste containing nano calcium carbonate can remineralize early lesions of enamel because nano calcium carbonate has good retention on enamel surface as it forms colloid particles followed by Ca2+ deposits. The minuscule particle sizes accelerate the dissolution of Ca2+ ions on nano calcium carbonate, thus increasing the concentration of Ca2+ ions and causing remineralization.[29]

Profilometer analysis has been used to measure the surface roughness of human enamel since it provides a relatively simple, sensitive, nondestructive and rapid quantitative method in demineralization and remineralization studies.[30] A pH cycling was performed in this study to simulate the remineralizing and demineralizing oral fluid conditions. Noninvasive treatment of WSLs by remineralization, represent a major advance in clinical management of the disease. Fluoride is considered from the best established remineralization strategy so it was chosen according to the finding which indicates that it is an efficient agent to aid remineralization and prevent demineralization of teeth, making it the reference agent against which new remineralizing agents are compared.[31]

In the present study there was a statiatical difference when comparing the mean surface roughness before and after applying MI paste Plus in treatment of WSL. These results come in agreement with a study made by Uysal et al.,[32] who reported that CPP-ACPF (MI paste Plus) was an effective treatment for remineralizing enamel lesions. Similarly, the studies of Srinivasan *et al.*[33], Wu et al.,[34] Robertson et al.,[35] Hamba et al.[36] and Jayarajan et al.[37] proved the efficiency of CPP-ACPF in WSLs remineralization. Again, in agreement with our study results, Shetty et al.[26] did an *invitro* study of 50 enamel samples, the results showed that there was improved enamel remineralization in the group, remineralized using CPP-ACPF in comparison with the other groups. However, in contrast with our results, a study made by Huang et al.[38] found that MI paste Plus did not appear to be an effective treatment for WSLs over 8 weeks. The authors claimed these results to the biased assessments due to lack of blinding to the evaluators as photographic assessment was used for WSLs clinical assessment.

Remineralization occurs when there are sufficient calcium and phosphate ions in the saliva to repair the dissolved hydroxyapatite structure, these agents can help the saliva in triggering remineralization. Noninvasive treatment of WSLs by remineralization, represent a major advance in clinical management of the disease. Fluoride is considered from the best established remineralization strategy. The remineralizing effect of mouthrinse may be explained by the fact that, in the presence of the Ca and PO_4 ions, which are produced during demineralization of the tooth enamel by acids, fluoride ions immediately promote the formation of FA or CaF_2.

Conclusion

Within the limitations of this in vitro study, the results showed that MI Paste Plus and NaF are effective for the treatment of WSLs. Although Kin toothpaste had an efficacy superior to the artificial saliva, its remineralizing effect seems to be inconsistent.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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