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Evaluation of Mechanical, Optical and Surface Changes of Pretreated Enamel Surface Bonded to Orthodontic Bracket Using Different Remineralizing Agents

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Abstract:

Objective: This study aimed to evaluate the effect of CPP-ACP, CPP-ACPF and nano-hydroxyapatite on enamel remineralization during fixed Orthodontic treatment. The treated specimens with different remineralizing agents were compared with their untreated and demineralized ones regarding surface roughness, surface microhardness, and color measurement.

Materials and methods: A total number of 120 specimens from extracted orthodontic patient teeth was collected and divided into 4 equal groups (10 specimens each) according to the enamel surface treatment applied immediately after PH cycling; group A received no surface treatment and served as a control group. In group B, the enamel surface was treated by CPP-ACP, while group C enamel surface was treated by CPP-ACFP, and group D was treated by Desensibilize NanoP. The surface roughness was obtained using surface Profilometer and Vickers microhardness was measured using Digital Display Vickers Microhardness Tester. Evaluation for each specimen was performed before, after pH cycling and after using different remineralizing agents. The color change was analyzed for each specimen in different groups under the Spectrophotometer. The change in color from control specimens compared to the color of the demineralized specimens (ΔE_1), the difference in color between control specimens and after remineralization (ΔE_2) and the change in color between demineralized and remineralized specimens (ΔE_3) were calculated. The results were then statistically analyzed and compared using ANOVA and LSD test at level of significance 0.05.

Results: The use of CPP-ACP, CCP-ACFP and NanoP significantly improved surface roughness, surface microhardness especially with NanoP with non-significant difference between the three remineralizing materials. NanoP exhibited the lowest change in color compared with CPP-ACFP and CPP-ACP paste.

Conclusion: The use of CPP-ACP, CPP-ACFP and NanoP could be used as preventive materials during orthodontic treatment and be effective in repairing the demineralized enamel restoring its surface smoothness, hardness, and color.

Keywords: Orthodontic brackets, Pretreated enamel surface, Remineralizing agents.

Introduction

Direct bonding of orthodontic brackets to enamel brought benefits to the orthodontist by simplifying and increasing the effectiveness of clinical procedures and to the patient by providing better esthetics and facilitated oral hygiene.[1,2] However, it causes an increase in white spots on the enamel surface adjacent to orthodontic brackets as a result of accumulation of biofilm around the brackets due to poor oral hygiene.[1] White spot lesion is defined as sub-surface enamel porosity from carious demineralization that presents a milky-white opaque color when located on smooth surfaces.[2]

Historically, topical fluoride application has been the most common method to prevent the development of white spot lesions around orthodontic appliances.[3]Fluoride application enables the formation of high quality fluorapatite that aids remineralization and inhibits glycolysis of plaque microorganisms. Other methods of fluoride administration have been investigated including bonding agents, cementing media.[4]

Casein phosphopeptide – amorphous calcium phosphate (CPP-ACP) complex was patented by the University of Melbourne, Australia, and the Victorian Dairy Industry Authority, Abbotsford, Australia.[5] The concept of using CPP-ACP for caries prevention was addressed in the eighties,[6] and the use of ACP technology started in the early nineties.[7]

The CPP-ACP is a milk-derived product that strengthens and remineralizes teeth and helps preventing dental caries.[8] It is composed of two-phase system which when mixed together reacts to form ACP material that precipitates onto the tooth structure, where it buffers free calcium and phosphate ion activities, maintaining a state of supersaturation, thus preventing demineralization and facilitating remineralization.[9] It is available in solutions, gums, lozenges and creams.[8]

Recent studies recognized that incorporation of fluoride into the CPP-ACP structure (CPP-ACPF) produced greater remineralization than the CPP-ACP alone.[10] The synergistic effect between CPP-ACP and fluoride which can be attributed to the formation of CPP-stabilized amorphous calcium fluoride phosphate, results in the increased incorporation of fluoride ions into plaque, together with increased concentrations of bioavailable calcium and phosphate ions.[11]

Hydroxyapatite (HA) is one of the most biocompatible and bioactive materials and is widely applied to coat artificial joints and tooth roots.[12] Nano-sized particles have similarity to the apatite crystal of tooth enamel in morphology, crystal structure and crystallinity.[13] In recent years, an increasing number of reports have shown that nano-hydroxyapatite has the potential to remineralize artificial carious lesions following addition to toothpastes or mouthwashes.[14]

Remineralizing agent is essential to protect the tooth from disadvantages of orthodontic treatment. However, it is unclear whether CPP-ACP, CPP-ACPF and nano-hydroxyapatite will influence the remineralization of tooth during orthodontic treatment. Therefore, the purpose of this study is to investigate to what extend could CPP-ACP, CPP-ACPF and nano-hydroxyapatite affect remineralization of teeth after orthodontic treatment regarding surface roughness, hardness, and color change.

MATERIALS AND METHODS

The materials used in this study were:

1. Casein phosphopeptide – amorphous calcium phosphate (MI Paste).
2. Casein phosphopeptide-amorphous calcium phosphate fluoride (MI Paste Plus).
3. Nano-hydroxyapatite (Desensibilize Nano P).

Specimen preparation

A total number of 120 specimens was collected from extracted human patient teeth whose treatment plan needs orthodontic extractions. The exclusion criteria for selection of the samples were the teeth with caries, cracks, erosion, fluorosis, hypo-calcification, and or restored teeth. The crowns were separated from their roots and sliced mesiodistally into buccal and lingual halves using a diamond disk bur.

Grouping of specimens

The prepared teeth specimens were randomly divided into 4 groups with respect to enamel treatment as follows:

Group A: Ten specimens were used as a control group (no enamel pre- treatment).

Group B: Ten specimens with their crown surfaces (buccal and lingual) treated with CPP-ACP.

Group C: Ten specimens with their crown surfaces (buccal and lingual) treated with CPP-ACPF.

Group D: Ten specimens with their crown surfaces (buccal and lingual) treated with nanohydroxyapatite.

pH-cycling model

The cycling schedule was designed to approximate the pH dynamics of the oral environment. Each cycle involves demineralization (pH 4.3) for 3hrs to simulate the daily acid challenges occurring in the oral cavity, followed by remineralization (pH 7.4) for 21 hrs. This process has been repeated for 12 days, then, specimens were kept for 2 more days in remineralizing solution. Teeth were rinsed with normal saline for 10s and wiped by tissue paper between demineralization and remineralization process, also at the end of pH-cycling protocol.

The demineralizing solution used contained 2.0 mmol/L calcium, 2.0 mmol/L phosphate in 75 mmol/L acetate buffer and the remineralizing solution contained 1.5 mmol/L calcium, 0.9 mmol/L phosphate and 150 mmol/L KCl in 20 mmol/L cacodylate buffer. There were many protocols in pH-cycling models, the protocol described by Featherstone et al and modified by Argenta et al,[15] was used in the present study.

Remineralization

All the used remineralizing materials were applied according to manufacturer's instructions. For CPP-ACP and CPP-ACFP pastes, pea size amount of the material was used to cover the specimens by cotton swab, followed by rubbing with finger for 4 minutes. The remnant was left for 30 minutes.[16] Finally, the remaining residue was washed with deionized water. It was recommended up to 2 weeks applications with an interval of 3 days. Regarding Nanohydroxyapatite, the product was applied with the aid of a micro applicator (Cavibrush - FGM), with a felt disk (wad) (Diamond Flex - FGM). The product was rubbed on the enamel surface for 10 seconds.[17]

The product was left in contact with the tooth for 5 minutes, then, excess of material was removed with a cotton ball. Finally, after 30 minutes, the specimens were washed with deionized water. It was recommended up to 2 weeks applications with an interval of 3 days. Material specimens contact and repeated application were standardized.

The specimens were subjected to the following tests before, after pH cycling and after using different remineralizing agents:

Surface roughness testing

A total number of forty specimens were prepared (10 specimens for each group) and measured using surface Profilometer (DEKTAK-3 version 2.13; Uberingen, Germany).[18] Surface roughness (Ra) was determined in µm using a diamond stylus tip (radius of 2 Nm) that was moved across the surface under a constant load with a speed of 0.25 mm/sec with a cut-off value of 0.8 mm. The average roughness value (Ra) was recorded for each specimen.

Surface microhardness testing

A total number of 40 specimens (10 specimens for each group) were measured using Digital Display Vickers Microhardness Tester (Model HVS-50, Laizhou Huayin Testing Instrument Co., Ltd. China) with a Vickers diamond indenter and a 20X objective lens to determine surface microhardness of enamel surface. A load of 200g was applied to the surface of the specimens for 10 seconds. Three indentations, which were equally placed over a circle and not closer than 0.5 mm to the adjacent indentations, were made on the surface of each specimen. The diagonals length of the indentations was measured by built in scaled microscope and Vickers values were converted into micro-hardness values.

Micro-hardness was obtained using the following equation:

$$\mathbf{HV=1.854\ P/d^2}$$

where, **HV** is Vickers hardness in Kgf/mm^2, **P** is the load in Kgf and **d** is the length of the diagonals in mm.[19]

Color change testing

Ten specimens for each group with a total number of 40 specimens were analyzed under the Spectrophotometer (UV-Shimadzu 3101 PC, Japan) to assess color changes of enamel surface. It consists of the photometer unit and a pc computer. To standardize the ambient light during the measurement process, specimens were mounted in a sample holder inside the light cabin of the apparatus.

The spectrophotometric readings of each specimen were recorded, and the color of the measured tooth specimen was measured on the CIE lab system as three coordinates L* a* b*color parameters. In the color space, L* is a measure of the Lightness of an object, ranging from 0 (Black) to 100

(White), a* is a measure of redness (a > 0) or greenness (a < 0) and b* is a measure of yellowness (b > 0) or blueness (b < 0). The difference between colors (ΔE) was obtained using the following formula,[20]

$$\Delta E = [(L_1-L_0)^2+(a_1-a_0)^2+(b_1-b_0)^2]^{1/2}$$

The change in color from control specimens compared to the color of the demineralized specimens was calculated (ΔE_1). The difference in color between control specimens and after remineralization was also calculated (ΔE_2). Finally, the change in color between demineralized and remineralized specimens was calculated (ΔE_3).

All data were analyzed using one-way ANOVA and LSD test at a level of significance 0.05.

RESULTS

Surface roughness

Mean values and standard deviations (SD) of surface roughness (µm) of the specimens before, after pH cycling and after remineralization using different remineralizing agents are shown in **Table 1**. A graphical presentation of these values is shown in **Figure 1**. Results showed that specimens mean values after demineralization increased significantly, while after remineralization values decreased significantly and still higher than their control one. The highest mean values in each group were for specimens after demineralization, and the lowest was for specimens after remineralization in each group. Between groups, the highest mean value after remineralization was for specimens treated with CPP-ACPF, and the lowest values were for specimens treated with CPP-ACP and nanohydroxyapatite.

The result of LSD showed that there was no significant difference in surface roughness between the different treatment groups (P > 0.05).

Table 1. Means, standard deviations and LSD of surface roughness (µm) of specimens before, after pH cycling and after using different remineralizing agents.

	Group A		Group B		Group C		Group D		P^1
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	
Control	.2523	.0023^A	.2531	.0003^A	.2531	.0003^A	.2530	.0012^A	0.478
After Demineralization	.2564	.0005^B	.2562	.0010^B	.2566	.0005^B	.2559	.0007^B	0.192
After Remineralization			.2533	.0009^A	.2534	.0007^A	.2531	.0006^A	0.731
P^2	0.002*		0.0003*		0.0004*		0.001*		

*Means with same superscript letters are not significantly different.

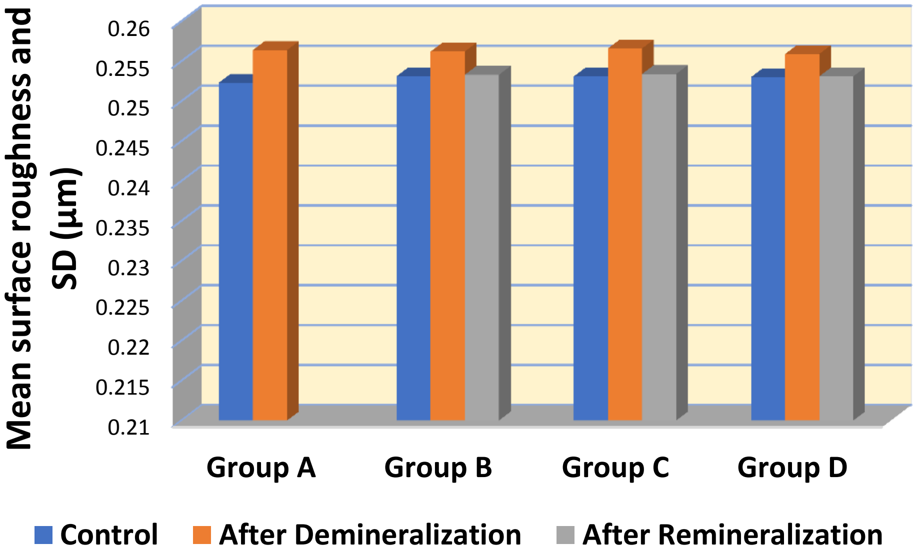

Figure 1. Bar charts for means and standard deviations of surface roughness of the specimens before, after pH cycling and after using different remineralizing agents .

Surface microhardness

Mean values and standard deviations of microhardness (Kgf/mm^2) of the specimens before, after pH cycling and after remineralization using different remineralizing agents are shown in **Table 2**. A graphical presentation of these values is shown in **Figure 2**. Results showed that specimens after demineralization showed a decrease in surface microhardness in all groups relative to the control groups.

After remineralization, group B exhibited the lowest microhardness values among all other treatment groups and in relation to the control group. Meanwhile, group D showed the highest microhardness value when compared to other treatment groups.

The LSD test showed significant difference among specimens after demineralization in relation to their control one and after remineralization when compared with their demineralized specimens ($P < 0.05$), while the difference was non-significant in all treatment groups in case of control specimens, after demineralization and remineralization ($P > 0.05$).

Table 2. Means, standard deviations and results of LSD of surface microhardness (Kgf/mm^2) of specimens before, after pH cycling and after using different remineralizing agents.

	Group A		**Group B**		**Group C**		**Group D**		
	Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**P^1**
Control	**255.97**	**17.33^{A}**	**257.49**	**31.55^{A}**	**259.85**	**19.29^{A}**	**263.60**	**9.58^{A}**	**0.860**
After Demineralization	**234.46**	**6.72^{B}**	**238.50**	**7.66^{B}**	**240.02**	**5.72^{B}**	**246.04**	**14.93^{A}**	**0.070**
After Reminerlization			**252.96**	**19.08^{A}**	**254.02**	**16.37^{A}**	**261.68**	**9.56^{A}**	**0.404**
P^2	**0.002***		**0.032***		**0.006***		**0.018***		

*Means with same superscript letters are not significantly different.

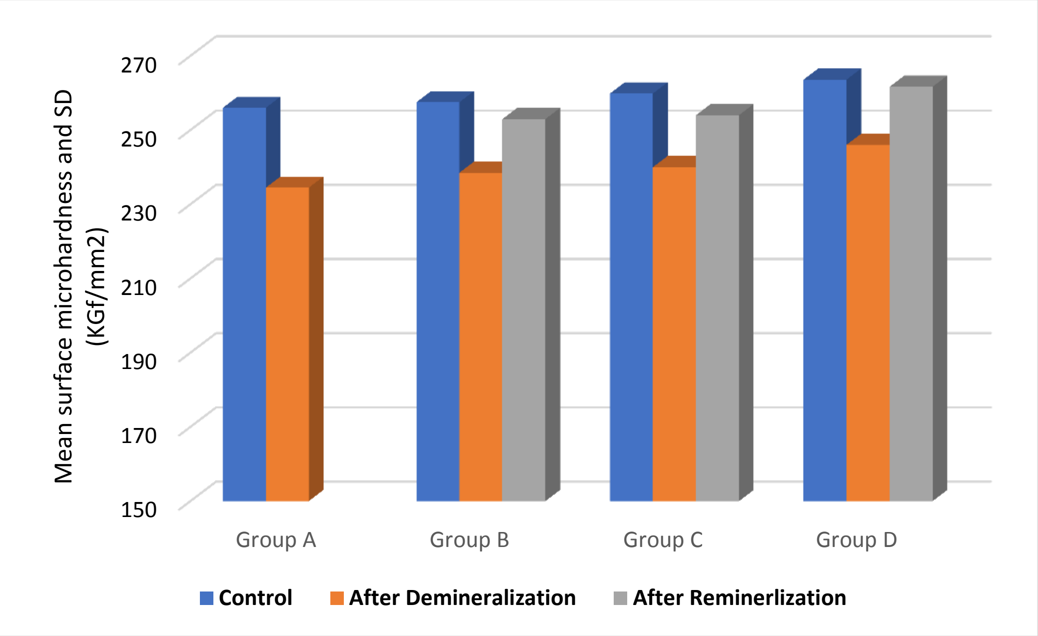

Figure 2. Bar charts for means and standard deviations of surface microhardness of the specimens before, after pH cycling and after using different remineralizing agents.

Color change measurement

Mean values and standard deviation of Color change (ΔE) between different treatment modalities among the study groups are presented in **table 3**. The change in color from the control specimens and after demineralization is denoted as ΔE_1, the difference in color between the control specimens and after remineralization is expressed as ΔE_2 and the change in color between demineralized and remineralized specimens is expressed as ΔE_3. A graphical presentation of these values is shown in **Figure 3**.

The color change (ΔE_1) at the baseline color measurement and after demineralization did not differ significantly between the treatment groups P = 0.26.

The (ΔE_2) of CPP-ACFP group, NanoP group and CPP-ACP group is presented in a descending order and showed no significant difference ($p > 0.05$).

After treatment application (ΔE_3), the change in color of WSLs was decreased in all treatment groups, while there was no significant difference between all groups ($P > 0.05$).

Table 3. Means, standard deviations and results of LSD test of color change (ΔE) between different treatment stages among the study groups.

	ΔE1		**ΔE2**		**ΔE3**	
	Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
Group A	**9.41**	**±3.94^{A}**				
Group B	**8.87**	**±2.50^{A}**	**8.36**	**±4.21^{A}**	**3.66**	**±0.23^{A}**

Group C	**11.14**	**$\pm1.69^{A}$**	**9.85**	**$\pm1.60^{A}$**	**3.62**	**$\pm0.15^{A}$**
Group D	**11.48**	**$\pm1.07^{A}$**	**9.22**	**$\pm0.65^{A}$**	**3.49**	**$\pm0.23^{A}$**
P	**0.26**		**0.5**		**0.28**	

*Means with same superscript letters are not significantly different.

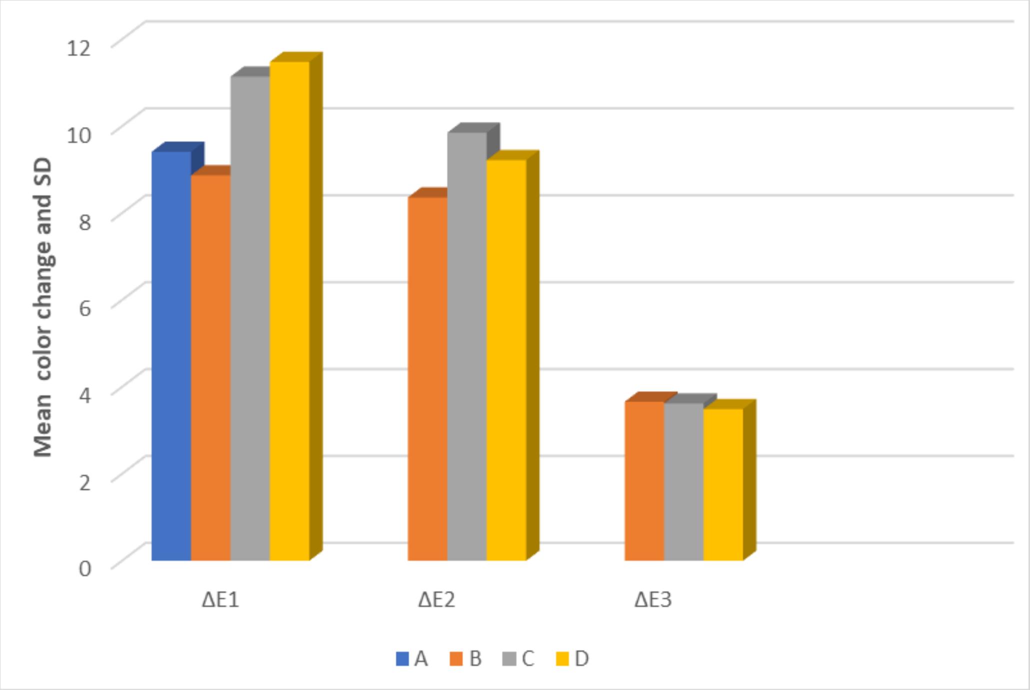

Figure 3. Bar charts for Means, standard deviations and results of LSD test values of (ΔE) between different treatment stages among the study groups.

DISCUSSION

In the present study, a demineralizing solution was prepared like a modification done by Argenta et al.,[15] to the methods described by Featherstone et al. The solution used in pH cycling was changed daily to avoid supersaturation.[17] pH cycling led to demineralization, significant increase in surface roughness, and significant decrease in microhardness indicating loss of minerals like calcium and phosphate. When minerals are lost from the enamel, it becomes more porous, the light is scattered, and the result is the clinical appearance of white spot lesions.[21]

Roughness measurements were performed using profilometry, which has an advantage of accurate and precise quantitative measurement without the need for additional measurements.[22]

After remineralization, roughness decreased significantly with remineralizing agents used in the present study in relation to their demineralized groups. The highest values were for specimens treated with CPP-ACPF; however, the roughness lowest values were for specimens treated with nanohydroxyapatite and CPP-ACP. This may be related to the different composition of each agent being formed from more than one ingredient and has its own manner in remineralization.[23]

The increase in the surface roughness after demineralization may be attributed to the dissolution of hydroxy apatite crystals present naturally in teeth.[24] Reduction in surface roughness after using CPP-ACP and CPP-ACFP may be due to their remineralization effect. This could be due to their nature that enables calcium and phosphate ions to be released from the remineralizing agent to infiltrate and diffuse surface and subsurface enamel porosities,[25] reduce the lesion depth, improve surface roughness and promote enamel remineralization.[26] These results agree with Al-Ani et al.,[28] who concluded that CPP−ACP tooth mousse improved roughness significantly after pH cycling of enamel.

Specimens treated with nanohydroxyapatite (Nano P) has a smother surface than CPP-ACFP. This may be due to the application form of Nano P paste (10 seconds of friction) according to the manufacturer's instruction that could lead to protective layer formation with globular deposits of nano-hydroxyapatite crystals.[17]

The result of our study agreed with de Carvalho et al,[17] who stated that CPP-ACFP paste treated surface enamel has no protective layer, while Nano-hydroxyapatite paste has a protective layer formation with globular deposits of nano-hydroxyapatite crystals by AFM observation.

The specimens showed a significant increase in microhardness in all tested groups after remineralization in relation to their demineralized ones. Nano-HAP group showed the highest mean microhardness values, followed by CPP-ACPF group and finally CPP-ACP group.

The changes of microhardness in CPP-ACPF group were in agreement with the results of Srinivasan et al,[29] and El-Zayate,[30] who showed that CPP-ACFP significantly remineralized softened enamel more than CPP-ACP. This may be due to the formation of fluorapatite which is highly resistant to acid dissolution.[31]

The highest increase in microhardness values exhibited by Nano-HAP may be attributed to the application method described by the manufacturer of this paste (10 sec. of friction) and the calcium nanophosphate crystals may have penetrated more deeply into the defects of the carious enamel forming a reservoir-like deposit of calcium and phosphate ions.[32] Also, the fluoride concentration in the nano-HAP paste is 10 times higher (9000 ppm) than that of the CPP-ACP paste (900 ppm) which may affect the remineralization process.[29]

Color changes was evaluated using Spectrophotometer which are the most accurate, useful, and flexible instruments for overall color matching in dentistry.[32]

A non-significant decrease in ΔE_3 value in Nano P group was noted in comparison to CPP-ACP and CPP-ACFP paste groups, indicating that nanohydroxyapatite was the most effective treatment for masking the whitish appearance of WSLs. This may be attributed to the fact that the nano-sized hydroxyapatite (< 100 nm) can penetrate the enamel pores and have a great similarity with the apatite crystal of enamel in morphology and crystal structure[33] All remineralizing agents used in the present study can cause a non-significant color change in demineralized enamel and resulting in a more acceptable overall esthetic appearance of teeth with white spot lesions.

The two experimental groups (MI Paste Plus and MI Paste respectively) showed a lower non-significant decrease in the change in color of WSLs (ΔE_3) in comparison to Nano P during the study. It has been stated that CPP-ACFP has a greater tendency to remineralize WSLs even more than CPP-ACP.[31] It is expected that remineralizing treatments help to prevent light scattering by filling the porous areas and improve the appearance of enamel. The current results agreed with Mohammed et al.,[34] who proved that CPP-ACFP showed the most significant color stability and staining resistance compared to Fluoride Varnish and CPP-ACP.

CONCLUSIONS

CPP-ACP, CPP-ACFP and nano hydroxyapatite improved surface roughness, surface microhardness, and surface irregularity of demineralized enamel surface particularly with nano hydroxyapatite. Nano hydroxyapatite was the most effective treatment for masking the whitish appearance of WSLs, although all the remineralizing agents used in the present study resulted in a more acceptable overall esthetic appearance of teeth with white spot lesions.

Conflict of interest

The authors confirm no financial and personal relationships with other people or organizations that could improperly affect this work. This study is self-funded by the authors.

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