Comparison of Casein Phosphopeptide Amorphous Calcium Phosphate Fluoride and Fluoride Varnish on Remineralization of Early Caries Lesions Around Orthodontic Brackets

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Abstract

Objectives: Orthodontic treatment increases the risk of caries and enamel white spot lesions incidence on the buccal surface of teeth. This study aims to compare the effect of Casein PhosphoPeptide Amorphous Calcium Phosphate Fluoride and fluoride varnish on remineralization of white spot lesions around orthodontic brackets.

Materials and Methods: In this in-vitro study, 60 premolars teeth were etched for 15 seconds. Then brackets were bonded with Relicane bond. All teeth were immersed in a demineralization solution for 4 days.

The lower part of the bracket was painted with nail polish to prevent the effect of a remineralizing agent to serve as a control group. The teeth were divided into 4 groups, Group 1: treated with fluoride varnish for 3 weeks (one-time application), Group2: treated with fluoride varnish for 6 weeks (two-time application), Group 3: treated with casein phosphopeptide and amorphous calcium phosphate with fluoride (CPP-ACPF) for 3 weeks (twice a day), Group 4: treated with CPP-ACPF for 6 weeks (twice a day). Remineralizing agent was applied on the occlusal of bracket for every tooth Area.

then the depth of specimens in treatment and control groups was measured by AutoCAD 2007. Pair-T test and repeated measured ANOVA were used for data analysis by SPSS 23.

Result: There was no statistically significant difference between each group of treatment. However, mean remineralization depth in group fourth and mean remineralization area in group three were higher than other groups.

Conclusion: Fluoride varnish and CPP-ACPF have the potential to treat the white spot lesion which is created during orthodontic treatment. According to less fluoride varnish use, it seems this substance is a better option in case of patient's cooperation is not good.

Keywords : *Tooth remineralization , Amorphous calcium phosphate, Fluorides, orthodontics*

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Introduction

A fixed orthodontic device can lead to the accumulation of plaque in the tooth surface which is the major cause of spot lesions after orthodontic treatment (WSL).[1-4]

Fluoride is one of the most common materials used topically for the treatment of WSL.[5]

Fluoride is available in various forms including mouthwash, toothpaste, tooth powder, gel and varnish that all of them are effective in WSL treatment. The advantage of fluoride varnish is not needing the patient's cooperation and could be used by the clinician.[4-8]

Fluoride causes hypermineralization in the enamel surface layer which prevents the Ca and phosphate penetration to the lesion.[2-8] The other concern is undesired effects like fluorosis and toxicity in high doses.[9]

Another material with anti-caries property is casein phosphopeptide and amorphous calcium phosphate (CPP-ACP) with dairy used.[9] This product derived from milk and suggested to prevent caries and enamel demineralization by releasing elements during the extension of caries and increase enamel remineralization. As a result, if it is used in the early stages of disease, it can stop caries and improve the affected areas.[6]

MI paste (GC America, Alsip III) is a new material with nanoparticle structure which is prescribed in recent years for WSL remineralization. The active constituent of this product are CPP that fixe and replace Ca, phosphate and fluoride in tooth surface and cause WSL deep remineralization.[2] In addition to the potential of all anti-caries material like fluoride, CPP-ACP is safe, tasty and tolerable by humans.[9]

Sampath et all shows that CPP-ACP increases remineralization on 40 orthodontic patients.[6] While Huang showed that varnish and Mi Paste have no special effect on WSL.[2]

Some studies showed that the simultaneous use of CPP-ACP and fluoride have a synergic effect on remineralization.[2] MI Paste Plus is a new product with this compound, 900ppm fluoride and CPP-ACP. Regarding the difference in fluoride products and CPP-ACP effects on WSL, this study is designed to compare the effect of fluoride varnish and Mi Paste Plus in WSL remineralization around the orthodontic brackets.

Materails and Methods

60 healthy premolars that were extracted due to orthodontics selected and kept in normal saline. All surface plaques were wiped by rubber cap and Pamis Powder. The place of the bracket on the tooth buccal surface was marked. Etching acid was placed for 15 seconds on the tooth surface and washed for 30 seconds with water. All brackets were bonded with Relicane bond (made in India, manufacturing RelX composites) and cured with Kerr light cure device (made in USA, Detroit Dental Manufacturing Company). It is necessary to mention that the bracket of all teeth were the same (Roth, Dentarum, Germany) and bonded by one operator. Then, all teeth were placed on demineralization solution (containing 2mmol/L calcium chloride+ 2mmol/L tri-sodium phosphate+75 mmol/L buffer acetate) with pH=4.6 for 4 days. Then, they were exited from the demineralized solution and washed with deionized water and divided randomly into four groups (15 per each group). The lower part of the bracket was covered with nail polish to prevent remineralization solution effect and remained on tooth until all teeth were cut. It is worth noting that the ethical aspect of the present study was approved by the Research Ethics Committee of Hamadan University of Medical Sciences, Hamadan, Iran (ID: IR.UMSHA.REC.1396.668).

Then fluoride varnish and MI Paste Plus (casein phosphopeptide amorphous calcium phosphate fluoride) were rubbed to the teeth according to the following protocol:

Method I: fluoride varnish was used on the buccal surface in occlusal of bracket once in three weeks according to producer instruction.

Method II: fluoride varnish was used on the buccal surface in occlusal of bracket twice in 6 weeks.

Method III: CCP-ACPF was used twice a day with a micro-brush according to the producer instruction on the buccal surface in the occlusal bracket for three weeks.

Method IV: CCP-ACPF was used twice a day with a micro-brush on the buccal surface of the occlusal bracket for 6 weeks according to the producer instruction.

All four groups were kept in separate containers containing synthetic salvia with the given formula (NaCl 0.4, KCl 0.4, CaCl2.2H2O 0.795, NaHPO4.2H2O 0.78, Na2S.2H2O 0.005, Urea 1.0, H2O 1.0) g/L in order to

simulate mouth condition.

After three weeks, teeth of the first and third groups were removed from synthetic salvia and their bracket was removed also. Then, they were cut vertically[5] by using a uniaxial cutting machine, made in Mashhad, Iran. Each cut was rubbed with sandpaper to reach 50-micron thickness. Each cut was evaluated under a polarized microscope (Dewinter technologies, Italy) with 40X magnification. The photo of each sample was captured and the mineralization depth and area in each tooth in both treatment and control sides were measured by AutoCAD 2007 software. Teeth of second and fourth groups were removed from the solution after 6 weeks, with the same cut and were observed under a polarized microscope. The enamel in one specimen of the third group broke during removing brackets and excluded from the study. Two specimens of the second group were broke with cutting machines and destroyed. Two specimens of the first group, three specimens of the second group, one specimen of the third group, and two specimens of the fourth group were destroyed during enamel sandpapering and excluded the study. Finally, 13 specimens remain in each group except group 2 with 10 specimens.

Statistical Analysis

A paired T-test was used for comparison of means of two outcomes (area, depth) in each method separately, and repeated measures ANOVA test (RM-ANOVA) was used for comparison of the mean of treatment and control between the four methods. All the analysis was performed by SPSS 23.

Results

In this study, two treatment methods were used for surface remineralization. Then teeth were cut and observed to measure the depth and extent of lesion remineralization under a polarized microscope. A figure of specimen of each treatment and control group is shown in Figure 1A to E.

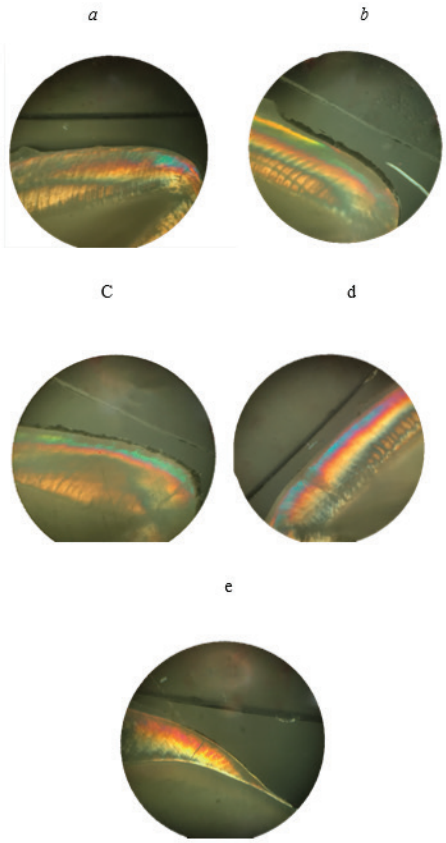

Fig. 1 a,tooth enamel 3 weeks after using fluoride varnish .b, tooth enamel 6 weeks after using fluoride varnish.c, tooth enamel 3 weeks after using CPP-ACPF.d, tooth enamel 6 weeks after using CPP-ACPF.e, An image of gingival part of bracket as control

Our result showed that without considering the four treatment methods, area and depth are significantly different in the two groups of control and treatment. (p<0.001) (Table1).

Table 1 The result of Paired t-test to compare area and depth of remineralization regardless of group

Group		N	Mean(SD)	T	p-value
Area	Treatment	49	0.252±0.29	5.873	<0.001
	Control	49	0.004±0.02		
Depth	Treatment	49	0.317±0.28	7.921	<0.001
	Control	49	0.007±0.03		

The result also shows that in each of the treatment method, area and depth were significantly different between the two groups of control and treatment (Table2). For example, the depth response in the first method for the treatment group is 0.298±0.312 And for the control group, it was 0.015±0.034 (Table2).

Table 2 The results of paired T-test and repeated measures ANOVA test.

Variable	Method	Treatment (SD)	Control (SD)	T-value	P-value*	P-value**
Depth(mm)	I	0.298(0.312)	0.015(0.034)	3.373	0.006	F=1.362, p=0.267
	II	0.276(0.295)	0.004(0.013)	2.875	0.018	
	III	0.307(0.217)	0.009(0.033)	5.371	<0.001	
	IV	0.376(0.306)	0.000(0.000)	4.436	0.001	
Area(mm2)	I	0.143(0.157)	0.013(0.030)	2.872	0.014	F=0.354, p=0.787
	II	0.262(0.322)	0.006(0.019)	2.464	0.036	
	III	0.362(0.402)	0.001(0.005)	3.252	0.007	
	IV	0.243(0.224)	0.000(0.000)	3.911	0.002	

***: Paired T-test statistic**

****: RM- ANOVA test statistic**

SD: Standard Deviation

I: fluoride varnish was used on buccal surface in occlusal of bracket once in three weeks according to producer instruction, ***II***: fluoride varnish was used on buccal surface in occlusal of bracket twice in 6 weeks, ***III***: CCP-ACPF was used twice a day with micro-brush according to the producer instruction on the buccal surface in occlusal bracket for three weeks, ***IV***: CCP-ACPF was used twice a day with micro-brush on the buccal surface of occlusal bracket for 6 weeks according to the producer instruction.

This result was also the same for other treatment methods. But the findings showed no significant difference between the depths of different treatment methods (p=0.267) and also between the area of different treatment methods. (p=0.787)

Discussion

Based on results, it is clear that four treatment methods have no statistical significant different in tooth surface remineralization and depth. However, mean remineralization depth in group four (treated with CPP-ACPF for 6 weeks (twice a day)) and mean remineralization area in group three (treated with CPP-ACPF for 3 weeks (twice a day)) were higher than other groups.

White spot lesions (WSL) which are visible clinically are seen in about 50% of orthodontic patients.[11] Different methods have been introduced to prevent WSL during orthodontic treatment. Among them laser, bonding material and fluoride-releasing composites, self-etch bonding, and fluoride releasing material and calcium depositing materials like fluoride varnish and CCP-ACPF can be referred to Miresmaeili et al.[12]

Advantage of fluoride varnish was adhesiveness to tooth surface, long-term fluoride release and ease of use (4-8). Todd et.al reported that fluoride varnish caused 50% reduction in demineralization compared to control group.[13]

Fluoride reduces demineralization which imposes this effect through antibacterial effect and producing hydroxyl apatite fluoride which is more resistant to acid.[14]

CPP-ACP is another remineralization method that in addition to anti-caries characteristics of fluoride varnish, its desired taste and odor can be optimal for patients.

The study of Srinivasan etal. on the remineralization potential of CPP-ACP toothpaste and CPP-ACP and fluoride toothpaste indicates higher remineralization in simultaneous use of both materials.[15]

Different direct and indirect methods like abrasion, scratching and indentation or atomic force microscopy and micro-hardness were used to evaluate demineralization and remineralization of enamel.[16] In this study, enamel lesion remineralization was measured by using polarized microscope. In method like indentation and scratching, only the instance surface is evaluated and the accurate amount of caries, remineralization and demineralization extension is not possible. In micro-hardness, a diamond blade entered into the sample and based on this, surface hardness was measured.[17] The amount of micro-hardness depends highly on experimental conditions and in addition of demineralization and remineralization, other factors are effective on it. Micro-hardness is a physical criterion which can be used to determine the tooth mineralization degree. This criterion is directly related to the arrangement of enamel crystals and its mineral content. [18] We can say that polarized microscope is the most objective method for measuring demineralization and remineralization.[15]

It can be said that the reason of significant statistical difference in N.Srinivasan study, compared with this study that results were not statistically different, was the difference in enamel remineralization measurement method that used micro-hardness.[15]

Jialing Li et.al concluded in a review study that CPP-ACP in the long-term has considerable effect on WSL but it has no significant difference compared to long- term effect of fluoride.[19]

In a study by Jayanth Jayarajan et.al study, the effect of CPP-ACPF on tooth remineralization was compared with CPP-ACP. Based on the results of this study, both materials had higher remineralization compared to synthetic salvia but CPP-ACPF had higher remineralization compared to CPP-ACP which is likely due to fluoride release.[20]

In this study, the mean remineralization area and depth difference in all treatment groups was significant compared with control group. In the current study, by increasing the use of fluoride varnish, the remineralization area increases which can be due two times of using fluoride varnish but mineralization area of CPP-ACPF, it has reduced which is likely due to the higher penetration of this material to the depth and increase in depth remineralization which is opposite to fluoride varnish feature that increases remineralization in the surface layer. The mineralized surface prevents the penetration of remineralization materials to depth.[2,8] Low mean remineralization depth in group 2 compared

to group 1 indicates this issue. It is worthy to mention that none of these differences were significant statistically.

Conclusion

Using fluoride varnish and CPP-ACPF can be effective in WSL remineralization. Regarding the lower number of fluoride varnish, compared to CPP-ACPF, it seems that varnish is a better option to prevent WSL.

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