

Remineralization Effects of Anticariogenic Products on Demineralized Enamel: an in Vitro Study.

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Abstract

Background/Objectives: The aim of this study was to compare the remineralization effects of two types of anticariogenic products on artificially demineralized enamel.

Methods/Statistical analysis: Extracted 60 premolars were decalcified in artificial acidic solution at 37°C for 7 days. Two anticariogenic products, Clinpro™ Tooth Cream (CTC) and Tooth Mousse-Plus® (TMP), were applied on demineralized surface for 3 weeks. The changes of surface microhardness values of each group were measured with Vickers surface hardness tester.

Findings: Microhardness values of CTC and TMP were increased during 3 weeks. Mean values of microhardness values of CTC was higher than TMP. Mean values of microhardness values was increased in 1st week < 2nd week < 3rd week (p < 0.05). There was a difference of significance between each week groups. Microhardness values of CTC was increased rapidly in 1st week. Then slower than 1st week during 2nd and 3rd weeks. TMP was increased slowly in 1st week then, faster in 2nd week. CTC was most effective during the initial week, and it was noted that a TMP could be expected to work if it was applied for at least two weeks.

Improvements/Applications: This has shown that if CTC and TMP are used for more than two weeks, it is valid for remineralization of the initial demineralized lesion.

Keywords: Casein phosphopeptide-amorphous phosphate, Functionalized tricalcium phosphate, Fluoride, Remineralization, Demineralization, Anticariogenic products.

1. Introduction

White spots are typical clinical symptoms of early dental caries and can be converted to normal through careful management [1]. Fluorine is commonly used as a preventive agent against dental caries. However, although used in appropriate quantities, the risk of excessive fluoride intake is remained. Recently, it is developed as an anti-cariogenic product containing components such as casein phosphopeptide-amorphous phosphate (CPP-ACP) and tricalcium phosphate (TCP) [2]. CPP-ACP, unlike fluoride, can be added to food, toothpaste, and tooth brushing solutions, slow down the progression of dental caries, and reduce the erosion of enamel surface [3, 4]. The Tooth Mousse-Plus® (TMP, GC Co., Tokyo and Japan) added 900 ppm of fluoride to the existing CPP-ACP preparation, which improved its anti-cariogenic effect [5]. On the other hand, Clinpro™ Tooth Cream (CTC, 3 M ESPE, Saint Paul, MN, USA) can be used like toothpaste and may be an

alternative as it contains functionalized TCP (f-TCP). However, there is not enough research yet. The purpose of this study was to compare the effects of remineralization of each fluoridation alternative material with different ingredients and application methods.

2. Materials and Methods

2.1. Research subject and method

In this study, Tooth Mousse Plus (TMP, GC Co., Tokyo, Japan) and Clinpro™ Tooth Cream (CTC, 3 M ESPE, Saint Paul, MN, USA) were used as anti-cariogenic products. Components of replacement of fluoride are described in Table 1. Immediately after the extraction of premolar, the tooth is stored in a 0.1 % Thymol solution [6]. They excluded those with dental caries, decalcification, erosion and fracture.

Table 1: Components of anticariogenic products and sample distribution

Group	Products	Manufacturer	Components	Sample No.
I	No Treatment			11
II	Tooth Mousse-Plus®	GC Co., Tokyo, Japan	900 ppm Fluoride, CPP-ACP	10

III	Clinpro™ Tooth Cream	3M ESPE, St. Paul, MN, USA	950 ppm Fluoride, fTCP	14
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CPP-ACP: casein phosphopeptide-amorphous phosphate
 fTCP: functionalized tricalcium phosphate

2.2. Decalcification on Enamel Surface

Artificial cariogenic solution for decalcification is consist of 1M lactic acid, 1% Carbopol C-907 (907, Noveon Inc., US), 50% NaOH, and 1.5 g HAP, 20% HCl. Thisis manufactured at pH 5.0[10]. The specimen was immersed in a manufactured artificial cariogenic solution and stored for four days to make decalcified lesion and keep in a constant temperature machine at 37 °C.

2.3. Artificial Saliva

Artificial saliva is manufactured with a pH of 7.0 by dissolving Xerova Solution (KOLMAR KOREA) in 300mL of the secondary distilled water[7]. Artificial saliva was replaced at intervals of one week.

2.4. Surface Micro-Hardness

The surface microhardness values were measured using a Vickers hardness tester (KM-122, Akashi, Japan). Surface microhardness values is indicated by the Vickers hardness number (VHN). The surface microhardness values were measured three times with a load of 50g for 10 seconds. The average of three of the values of each specimen is obtained.

2.5. Specimen Classification

The teeth were intended for extraction for orthodontic need and were collected with informed consent from the patient. A total of 60 premolar teeth with surface microhardness values of 270 - 300 VHN were prepared. They were divided into three groups of 10. Of these, specimens within the range of 10-30 VHN were selected after the formation of the initial decalcification. Each specimen was classified as Group I is No treatment, Group II is TMP and Group III is CTC. The number of each group is described in Table 1.

2.6. Application of Anti-Cariogenic Products

Group I replaced the artificial cariogenic solution every week, without any treatment. The group II used a glass plate to apply anti-cariogenic products for three minutes, and then washed them with distilled water every 12 hours. The group III was applied with a toothbrush for two minutes every 12 hours and then cleaned

with distilled water.

2.7. Statistical Analysis

It was analyzed using SPSS 23 (SPSS Inc., Chicago, IL, USA) and Excel 2007 (Microsoft Inc., Chicago, IL, USA). Wilcoxon-Signed Rank tests were used in each group to determine the difference in time change. Kruskal-Wallis test and Mann-Whitney test were used to compare the significance differences between the groups. The level of significance was $p < 0.05$.

3. Results and Discussion

There was an increase in surface microhardness values in both group. There was a significantly higher remineralization effect in group III than in group II. In the first week, the surface microhardness values of the III group increased dramatically ($p < 0.05$), and in the second and third weeks, the rate of increase was somewhat reduced as shown in figure 1.

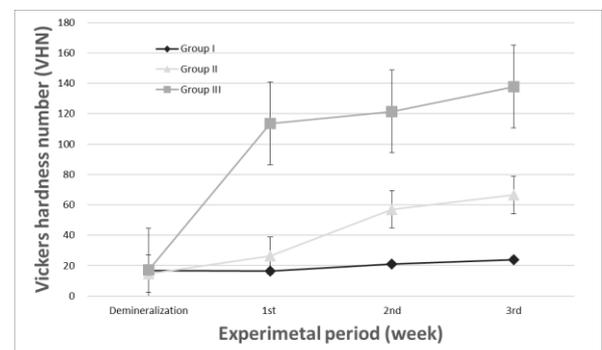


Figure 1: Changes of surface-microhardness for a period of 3 weeks.

The measuring of surface micro-hardness to compare changes over time were made on 7 days, 14 days, and 21 days after the artificial demineralization. The average of the surface microhardness values for each product was 20.9 ± 1.7 for the I group, 57.0 ± 5.1 for the II group, and 119.3 ± 6.8 for the III group. The III group was the highest. The average values of microhardness according to the period of application for each product are shown in Table 2.

Table 2: The mean of surface microhardness(VHN) among groups

Group	SOUND (VHN)	Demineralization (VHN)	1 st week (VHN)	2 nd week (VHN)	3 rd week (VHN)
I	304.1 ± 30.5	16.8 ± 11.9	16.0 ± 8.34*	21.9 ± 13.5*	24.7 ± 12.7*
II	315.2 ± 37.2	19.5 ± 21.5	35.5 ± 32.2*	68.2 ± 38.1*	67.4 ± 25.4*
III	308.0 ± 31.0	22.7 ± 16.7	108.3 ± 49.7*	117.6 ± 45.2*	132.2 ± 45.4*
P < 0.05		P= 0.149	P= 0.000	P= 0.002	P= 0.000

P value = Kruskal-Wallis Test ($p < 0.05$)

*: P value by Mann-Whitney Test.

There was a statistically significant difference between groups I, II and III ($p < 0.05$) as shown in Table 3.

Table 3: Statistical significance of surface microhardness(VHN) among groups

Group	I	II	III
I			
II	+		

III	+	+	
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P value = One-way Anova and Tukey test ($p < 0.05$)

The rate of surface microhardness recovery was calculated using the method suggested by Gelhard[8]. The change in surface hardness recovery rates for the first week in Table 4 was 3.38% in group II and 33.68% in group III. In the second week, the group II showed 14.28% and the group III 36.83%, while in the third week,

the group II showed 17.51% and the group III 42.88%.

Table 4: comparison of the percentages of surface microhardness recovery, or SMHR

Group	1 st week(%)	2 nd week(%)	3 rd week(%)
I	-0.18 ± 0.94	1.54 ± 2.44	2.40 ± 4.47
II	3.38 ± 6.65	14.28 ± 12.25	17.51 ± 10.78
III	33.68 ± 22.81	36.83 ± 22.28	42.88 ± 26.94

SMHR: surface microhardness recovery

Remineralization is defined as the process of producing minerals by supplying calcium and phosphoric ions to an empty space in demineralized enamel[9]. In proper oral conditions, saliva, including minerals, can lead to remineralization, which can change the procedure of carious processing. Fixed braces interfere with cleaning and physical removing of plaque by making it difficult to brush. As a result, oral hygiene is bad, and demineralization is carried out. Demineralization is one of the common negative side effects of orthodontic treatment. [10, 11].

Fluoride has been the most widely used so far in the prevention of tooth demineralization and remineralization of initial dental caries. However, this method requires regular fluoridation by experts and has possibility of toxic effect in case of overuse[12]. CPP-ACP and TCP, f-TCP components are alternatives to fluoride, which induces remineralization[9]. The CPP-ACP, a milk protein extract, has buffering function for the activities of calcium and phosphoric acid. It has anti-cariogenic effect by reducing the rate of demineralization and enhancing remineralization[3]. Recently, it has been reported that fluoride added to CPP-ACP has a synergy effect on the remineralization of early cavities[13]. As another alternative, f-TCP is the functionalized β -TCP. It was produced by mechanical reaction of β -TCP with sodium lauryl sulfate or fumaric acid[14].

The study compared the remineralization effects of two anti-cariogenic agents that differ in their composition and application. The two agents were the Tooth Mouse Plus containing CPP-ACP with fluoride and Clinpro™ Tooth Cream containing f-TCP. CTC, which includes fluoride ions, has been reported to increase fluoride absorption, thus increasing remineralization[14]. TCP with fluoride is found to be effective than CPP-ACP with fluoride[15]. In the present research, group III was the highest surface-microhardness in every week for 3 weeks.

Surface microhardness values was increased in all groups where anti - cariogenic agents were applied. The average surface microhardness values for each group was higher in the group III than group II. In the first week, the surface microhardness values of the group III increased dramatically and continued to increase in the second and third weeks, but the increase rate was somewhat reduced. The surface microhardness values of the group II increases at 2nd week faster than 1st week. But the surface microhardness values were lower than the group III.

Some researchers reported that CPP-ACP with fluoride is less efficient than f-TCP[16]. The study also showed that the change in surface hardness recovery rate for the first week was 3.38% for the group II, and 33.68% for the group III, which was excellent in the group that contained f-TCP. These results can be inferred that the combination of f-TCP and fluoride has better remineralization effects than the combination of CPP-ACP and fluoride. However, another study evaluated the mineral loss using QLF-D (Quantitative Light-induced Fluorescence-Digital), and the CPP-ACP was smaller than the f-TCP[17, 18]. These results are contrary to the results of this study.

In the second week, the group II showed a greater increase than the one-week increase, while the group III showed a relatively small increase. This aspect was seen in 3 weeks and is therefore recommended to be applied for at least 2 weeks for the effect of CPP-ACP with fluoride. During the three-week experiment, the group III recovered 42.88% to determine that the f-TCP showed good remineralization efficiency.

4. Conclusion

The results of this study show that TMP and CTC are effective in remineralization of the artificially decalcified enamel. CTC, which showed a significant increase in surface microhardness values from the first week of application, will have a good remineralization effect in clinical practice. TMP was thought to be helpful if used continuously for more than two weeks.

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