Does Casein Phosphopeptid Amorphous Calcium Phosphate Provide Remineralization on White Spot Lesions and Inhibition of *Streptococcus mutans*?

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***Objective**: The aim of this study was to evaluate the remineralization effect of Casein Phosphopeptid Amorphous Calcium Phosphate (CPP-ACP) on white spot lesions (WSL) and its inhibitory effect on Streptococcus mutans colonization. Study design: The study group consisted of 60 children exhibiting at least 1-WSL. Subjects were randomly divided into 2 groups: a test group of using CPP-ACP cream (Tooth Mousse, GC Europe N.V., Leuven, Belgium) and a control group using only fluoride containing toothpaste for a period of 3-months. Baseline WSLs were scored using DIAGNOdent device (KaVo Germany) and the saliva samples were collected to measure S. mutans counts. After the 3-month period the WSLs were again recorded and the saliva sample collection was repeated. Wilcoxon Signed Ranks Test was used for statistical analysis. **Results**: DIAGNOdent measurements were increased by time (p=0.002) in control group and no statistically significant difference (p=0.217) was found in test group by the 3-month period. In both groups, the mutans counts were decreased in 3-month experimental period. **Conclusions**: These clinical and laboratory results suggested that CPP-ACP containing cream had a slight remineralization effect on the WSL in the 3-month evaluation period however longer observation is recommended to confirm whether the greater change in WSLs is maintained.*

Key words: Remineralization, DIAGNOdent, CPP-ACP

INTRODUCTION

Dental caries is one of the most common and preventable diseases of childhood. The process of caries formation is a cycle of remineralization and demineralization with various stages being either reversible or irreversible. White spot lesions (WSL) are manifestations of the earliest stage of caries progression and are capable of being reversible.[23] These lesions are characterized by a white, chalky, opaque appearance and are commonly located in pits, fissures, and smooth surfaces of teeth. The most frequently detected localization of WSLs is on the cervical third of the tooth. The WSLs can be spotted by many methods using a range of relatively new detection systems, including laser fluorescence, digital imaging fiber-optic transillumination, and quantitative light-induced fluorescence. These techniques are considered as possible supplemental techniques for detecting incipient carious lesions.[25] The DIAGNOdent (KaVo, Germany) is a laser fluorescence caries detection device that operates by illuminating a tooth surface with pulses of red laser light and then analyses the emitted fluorescence. Changes in the mineral content result in changes in patterns of fluorescence.[10,13] A numerical value is assigned to the degree of fluorescence, which is used to indicate the extent of caries.

The non-invasive treatment of early caries lesions by remineralization is a major advantage in clinical management and researchers have investigated the low cariogenic potential and possible cariostatic activity of dairy products such as milk, casein, caseinates and cheeses. Recently, casein phosphopeptide amorphous calcium phosphate (CPP-ACP) derived from milk protein casein has been reported to reduce demineralization of the tooth structure and enhance remineralization. The anticariogenic potential and remineralizing effects have been shown in *in vitro* and *in situ* studies.[5,8,9,13,14,15,16,17,18,19,26] CPP contains the cluster sequence of Ser (P)-Ser (P)-Ser (P)-Glu-Glu from casein.[9,21] and CPP-ACP is reported to have topical anticariogenic effects due to its ability to stabilize calcium and phosphate in an amorphous state. In acidic environment, ACP will separate from CPP, thereby increasing salivary calcium and phosphate levels.[14] Moreover, CPP can stabilize the level of ACP in saliva by preventing precipitation of calcium and phosphate, and stabilize the level of calcium. It can be anticipated that routine use of CPP-ACP cream would prevent WSL

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formation and remineralize present lesions. Therefore, the aim of this clinical study was to evaluate the remineralizing effect of a topical remineralizing cream containing CPP-ACP on WSLs and its inhibitory effect on *Strepteccocus mutans (S. mutans)* colonization and the tested hypothesis was that the use of CPP-ACP would have a remineralizing effect on WSLs and inhibiting effect on *S. mutans*.

MATERIALS AND METHOD

Approval for the clinical trial was obtained from the Human Ethics Committee of the University of Ege, City of Izmir, Turkey. Sixty healthy students of both sexes with no complicating medical history exhibiting at least one white spot lesion on their permanent upper central incisors or canines were invited to participate the study. The children who had taken antibiotics within the last month and milk protein allergy were excluded. The mean age of the children was 13±0.68 years and the mean DMFS score was 1.95±2.22.

The subject flow in the study is summarized in Figure 1. The subjects were randomly divided into 2 groups: a test group of 29 patients using Tooth Mousse, (GC Europe N.V., Leuven, Belgium) (TM group) and a control group with 31 patients using routine fluoride toothpastes (Colgate, 1450 ppm F, Colgate-Palmolive Company). Oral health education was provided to both groups at baseline and 3 months.

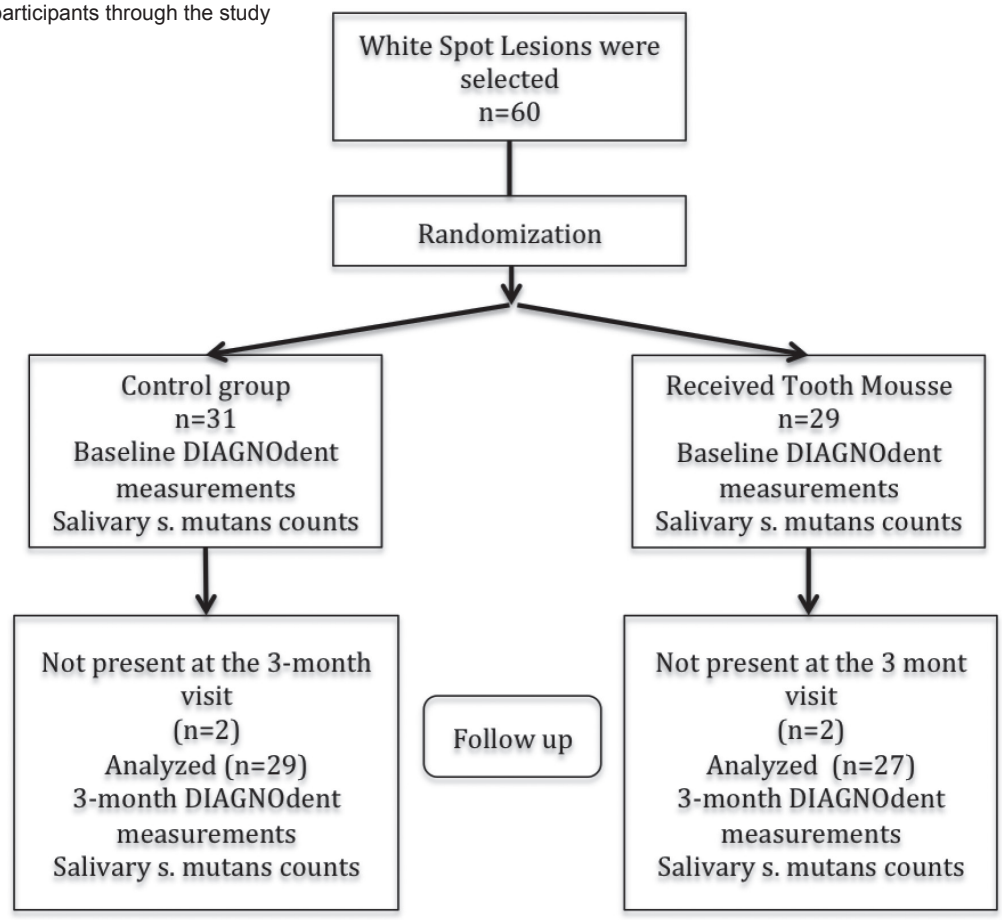
The control group were informed and encouraged to use a fluoride toothpaste (Colgate, 1450 ppm F) two times a day during 3 months. The test group was instructed to apply a pea-sized amount (approx. 1g) of CPP-ACP containing cream. They advised to use the cream on the teeth twice a day after brushing his/her teeth with the same fluoride toothpaste (Colgate, 1450 ppm F) for a period of 3 months. Eating and drinking were prohibited for 30 minutes after application. The products were distributed to the subjects at baseline and they were asked to suffer from additional preventive measures based on fluoride during the experimental period.

The teeth were isolated with sterile cotton rolls and the surfaces of the teeth were cleaned with sterile gauze pads and all of the upper incisors and canines of each participant were examined for the presence of white spot lesions. All the determined lesions were recorded and the baseline WSLs were scored by using DIAGNOdent device, which was calibrated against its own ceramic standards according to the manufacturer's instructions before every use. The assessments were carried out by the same dentist who was blind as to group allocation of the subjects in all visits. The whole labial (buccal) surface of the tooth with a WSL was scanned. The labial window area was carefully scanned using the type B probe by holding the tip in close contact with the tooth surface and tilting the tip around the measuring site in order to collect the fluorescence from all directions.

After the 3-month experimental period the WSLs were again recorded using the DIAGNOdent device and the changes in the amount of the values and scores were recorded.

The unstimulated saliva samples were collected for 5 minutes from each participant at baseline and salivary flow rate was measured. The salivary samples were then transferred to the microbiology

Figure 1. Flow of participants through the study



laboratory for *S. mutans* counts and for the measurements of pH levels of the saliva, the sample collections were repeated at the end of the 3-month experimental period.

The samples were vortex-mixed to disperse bacterial aggregates and 0.1 ml of the samples were inoculated onto Mitis Salivarius Agar (MSA, Difco, Detroit, Mich, U.S.A.) supplemented with 15 % sucrose and bacitracin (0.2 U/ml) (Sigma Chemical Co., St. Louis, Mo., U.S.A.) for the selective isolation of *S. mutans*.[8] Two to three isolates representing colonial types of *S. mutans* were subcultured for each sample, and biochemically differentiated by colony morphology, the presence of catalase,[6] fermentation of inulin, mannitol, melibiose, raffinose, sorbitol, production of acetoin and dextran, hydrolysis of arginin and esculin.[12] Counts below 10 colony-forming units (CFU) were beyond detectable limits and recorded as 0 (not detectable).

Statistical analysis

All data were processed by SPSS software, (Version 19,0, SPSS Inc., Chicago, IL, USA). The differences in the groups at baseline and 3 month interval were calculated by Wilcoxon Signed Ranks Test. Mann Whitney U Test was used to determine statistically significant differences between both groups at baseline and 3 month interval. The level of significance for all tests was set at 5%.

RESULTS

The baseline salivary flow rate, salivary pH, DIAGNOdent measurements and salivary mutans values were not significantly different between the groups and were shown in Table 1.

Table 1- The baseline salivary flow rate, salivary pH, DIAGNOdent measurements and salivary mutans values of both groups (mean±SD)

	Salivary flow rate (ml/min)	Salivary pH	DIAGNOdent readings	Salivary mutans values CFU/ml
Control	0.61±0.31	6.92±0.26	14.14±10.32	$8.91x10^6$
Tooth Mousse	0.55±0.26	6.91±0.23	14.31±8.34	$10.18 x10^6$

DIAGNOdent readings increased in the control group after 3-months period and were shown in Figure 2 and this increase was found to be statistically significant (p=0.002). There was a decrease between the baseline and 3-month results of the DIAGNOdent measurements in the test group, but this was not statistically significant (p=0.217). Photographs of two patients who were in the test group were presented in Figure 3 and 4.

Microbiological analysis

The *S. mutans* levels in the saliva at the baseline and 3-month periods were shown in Figure 5. In the both control and test groups, the mutans counts in the saliva were decreased in 3-month experimental period, which was found to be statistically significant (p=0.000 for both groups).

Figure 2. Diagnodent measurements, (*) presents statistically significant difference

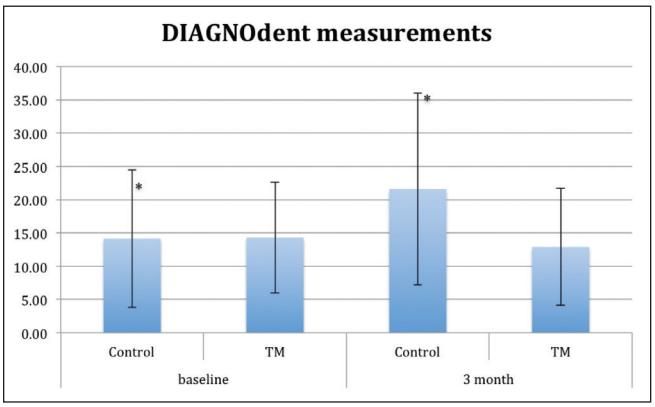


Figure 3. Intraoral photographs of a patient a) white spot lesions were seen at baseline b-c) at 3-month evaluation period after the application of Tooth Mousse

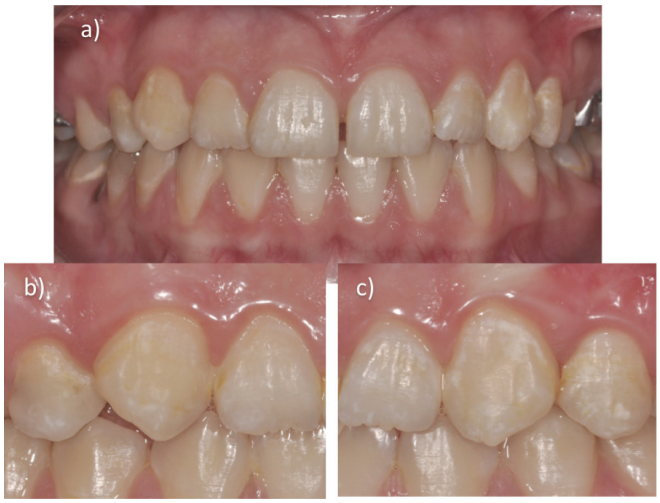


Figure 4. Intraoral photographs of a patient a) at baseline b) after the 3-month evaluation period after using Tooth Mousse

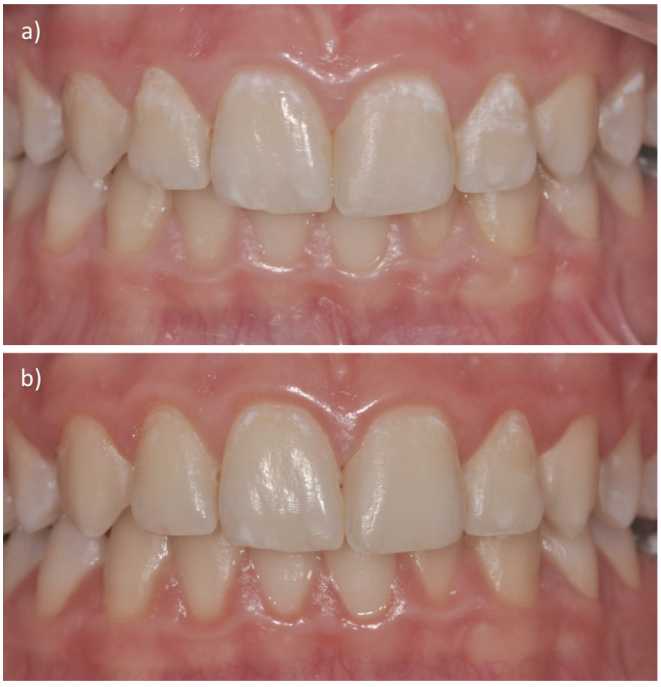
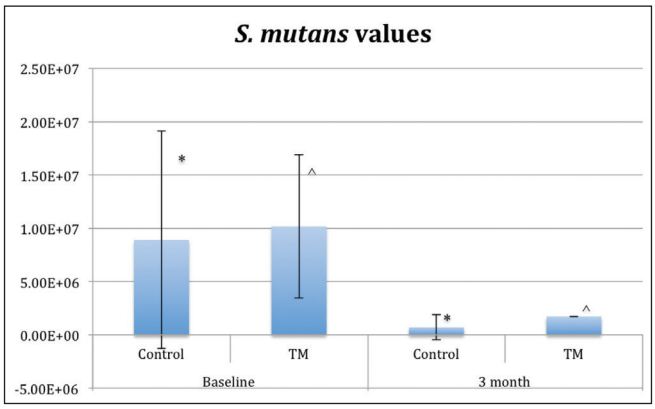


Figure 5. *S. mutans* levels in the saliva at the baseline and 3-month periods, (*,^) presents statistically significant difference in groups



DISCUSSION

This study evaluated the remineralization efficiency of a 3-month home application of CPP-ACP on white spot lesions and the inhibitory effect on *S. mutans* in comparison with a control group.

White spot lesions, which are the earliest phase of the caries process and which are reversible[23] can be treated by conventional approaches involving the disadvantage of being invasive.[11] Therefore, many remineralization agents can be used to promote remineralization by ionic exchange mechanism instead of invasive techniques. In the current study, CPP-ACP containing product is used, which has been reported to have the potential in promoting remineralization[5,9,14,15,18,21] and which maintains calcium and phosphate at a super saturated status compared to calcium and saliva and preserves them in close proximity to the enamel lesion, thereby decreasing demineralization and enhancing remineralization of enamel lesions.[19]

In this study, laser fluorescence system was used to detect the WSLs. The patented laser fluorescence method of DIAGNOdent works on the basis of the differing fluorescence between healthy dental substance and diseased dental substance and it detects even the smallest lesions; using a number scale and an audible alert. It has an acceptable sensitivity and specifity, which was reported before as compared with quantitative light-induced fluorescence.[22] Similarly, high sensitivity and acceptable specificity were also observed in other studies, such as those by Rocha *et al*[20] and Anttonen *et al*[2]. DIAGNOdent is considered to be useful and reproducible method that could be used to quantify WSLs and test the effectiveness of the preventive strategies.

In the present study three months after the application of CPP-ACP in vivo, DIAGNOdent measurements exhibited a slight decrease in the demineralization process in comparison with the control group. The slight decrease in DIAGNOdent readings for the WSLs in TM group compared to that of control group may be taken to indicate a reversal of the early caries process due to the usage of CPP-ACP might have taken place showing that new hydroxyapatite crystals were formed from the minerals existing in the paste by physical diffusion of the ions to the destroyed hydroxyapatite. The slight decrease in DIAGNOdent readings of TM group might also be explained by the changes in the surface properties of TM applied white spot lesions, like the changings in the porosity of enamel because the main function of casein phosphopeptides is to modulate bioavailability of calcium phosphate levels by maintaining ionic phosphate and calcium super saturation to increase remineralization however longer observation is recommended to confirm whether the greater change in WSLs is maintained. Andersson *et al*[1] reported a significant reduction in WSLs after the application of CPP-ACP at 12-month follow up as we expected. In a systematic review, Yengopal and Mickenautsch[24] reviewed the short-term remineralization effect of CPP-ACP in clinical *in situ* trials, and long-term caries-preventing effect for CPP-ACP *in vivo* in a randomized control trial. According to our results, CPPs could be suggested as an alternative preventive system against demineralization of early enamel lesions as published before.[16,17]

Our results indicated that the numbers of *S. mutans* decreased after the 3-month evaluation period in TM group. CPP also is believed to have an antibacterial and buffering effect on plaque and interfere in the growth and adherence of Streptococcus species. In the current study, we cannot correlate the reduction in *S. mutans* values only to the inhibiting effect of CPP-ACP because there was a significant decrease in the control group, too. In an *in vitro* study of Erdem *et al*, it was reported that there was a reduction in bacterial viability of *S. mutans* in biofilm after the application of CPP-ACP but this reduction was not statistically significant.[7] These reductions in both groups might also be related to the changes in oral hygiene behaviors of the children, because all of the children received oral health education at the beginning of the study individually. It could be suggested that children in both groups got more aware of their oral health care and followed the oral hygiene instructions more and consumed less cariogenic food in comparison with before.

The children used the CPP-ACP containing paste just after a routine fluoride containing toothpaste because of the ethical reasons and it is reported that fluoride also has a tendency to interact with the ACP component of the casein complex and may precipitate out as calcium fluoride, leading both inorganic components ineffective.[3] But this might happen only if massive excess of fluoride exists, because there is an also fluoride containing CPP-ACP product which contains 900 ppm of fluoride and Reynolds observed that a dentifrice containing 2% CPP-ACP plus 1100 ppm F was superior to all other formulations of mouthrinses and dentifrices containing CPP-ACP and fluoride.[16] Controversially in a study of Beerens *et al*,[4] who evaluated the effects of CPP-ACPF paste on dental plaque and on the remineralization of enamel WSLs after the removal of fixed orthodontic appliances during a 3-month time period, it was reported that there were no differences between the commercially available CPP-ACFP paste and the fluoride free control paste on the remineralization of enamel WSLs and plaque composition.

CONCLUSIONS

It can be concluded that within the limitations of this study CPP-ACP containing remineralizing paste had a slight remineralization effect in the 3-month evaluation period and longer observation period with an enlarged sample size is recommended to confirm whether the greater remineralization in early caries lesions is maintained.

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