

Effect of Different Topical Agents on Remineralization of Early Enamel Lesion– an *in vitro* Study

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Abstract

Objective: This study aimed to compare the effect of using Casein phosphopeptide – amorphous calcium phosphate (CPP-ACP) paste, Remin-Pro and Fluoride Varnish on remineralization of enamel lesions.

Methods: In this experimental-*in vitro* study, 60 intact premolars and molars were used and flat enamel surfaces were prepared. The specimens were divided into 6 groups (N=10). After primary DIAGNOdent value measurement and a four-day immersion in demineralizing solution, the DIAGNOdent value were measured. Groups 1, 2 and 3 were treated by Fluoride varnish, CPP-ACP and Remin-Pro respectively, according to the manufacturer instruction and their DIAGNOdent value was read. Groups 4, 5, and 6 were treated by Fluoride varnish, CPP-ACP and Remin-Pro for 1 month (8 hours a day), respectively, and their DIAGNOdent value was measured. Then the specimens of these three groups were demineralized and pH cycled and their DIAGNOdent values were recorded. The data were analyzed by One-way analysis of variance (ANOVA) and repeated measures ANOVA.

Results: After a one-month treatment, the DIAGNOdent value significantly decreased in groups 4, 5, and 6 in comparison to the manufacturer instruction ($p<0.001$). ANOVA test indicated that decrease mean value of DIAGNOdent value was significantly higher for Remin-Pro and CPP-ACP groups than Fluoride varnish group, from entrance time to the study to re-demineralization stage ($p<0.001$).

Conclusion: All the three materials showed a statistically significant amount of remineralization after repeated application but the CPP-ACP and Remin pro were more resistant to redemineralization and pH cycling.

Key words: CPP-ACP, Demineralization, DIAGNOdent, Fluoride varnish, Remineralization, Remin-Pro.

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Introduction:

Tooth structure in oral environment is exposed to frequent demineralization and remineralization and if the balance is lost for any reasons, it leads in destruction of dental structure (1). Primary enamel lesions are able to remineralize, especially using boosting

remineralization treatment (2).

Several studies have indicated that milk and its derivatives, such as cheese, has anti-caries properties in human beings and animal models, its functional mechanism is due to chemical effects of phosphor protein casein and calcium component of cheese (3). Casein phosphopeptide amorphous calcium phosphate, briefly called

CPP-ACP, has anti-caries protective effects through inhibition of demineralization and a combination of increase in remineralization and decrease in demineralization (1,4-7). Every functional potential of CPP-ACP is similar to the effects of the most common anti-caries substance, i.e. Fluoride. In addition, CPP-ACP is safe for the teeth, tastes good and is tolerated well by the patients. Unlike Fluoride, swallowing any amount of this substance is harmless and does not have any undesirable effects like Fluoride overuse (8-10). Accordingly, it seems that if the substance sufficiency for remineralization and inhibition of demineralization of tooth structure is proven, it can be a proper alternative to different kinds of Fluoride compounds.

Remin-Pro is also a new product. It is claimed that it prevents demineralization and erosion of tooth structure. Remin-Pro aids neutralization of acids in plaque, therefore it balances oral Flora. Fluoride and Hydroxyapatite in this material reinforce remineralization and strengthen enamel surface, as Remin-Pro is a novel sanitary product, few studies are conducted on its effect and on its comparison to Fluoride; more research is required to confirm the claim of the manufacturer (11).

Thus, it is intended to apply laser fluorescence technology to investigate the effect of preventive materials on remineralization of enamel lesions (12). DIAGNOdent (Dd) (KaVo, Biberach, Germany) is an instrument developed as an aid to early detection of caries lesions, and as a tool to monitor lesion progression over time, as well as to evaluate the outcome of preventive treatment (13, 14).

This study aims to compare the effect of CPP-ACP paste (GC tooth mousse, Japan), Remin-Pro (VOCO, Germany) and Fluoride varnish (Sodium Fluoride Sultan, USA) on remineralization of enamel lesions, using DIAGNOdent following demineralization through similar process to caries in oral

environment.

Methods:

Sixty intact human premolars extracted for orthodontic reason were collected and stored in distilled water until use (maximum one month). The samples that showed no evidence of white spot lesion, enamel cracks, or caries on visual inspection were evaluated.

The specimens were randomly divided to 6 groups (n=10). The randomization procedure was carried out by using sequentially numbered opaque sealed envelope prepared with unrestricted (simple) randomization. Each treatment agent was written and sealed in envelopes before beginning the study. The operator who carried out all the treatment opened an envelope for each case at the beginning of the treatment. Baseline primary DIAGNOdent value was measured in the center of polished section of every specimen by DIAGNOdent pen (DIAGNOdent pen 2190, SN 2002169, Germany).

The specimens were placed in incubator (37°C) in demineralizing solution for 4 days (96 hours). The solution was prepared using the formula Fluoride (0.2 ppm), Lactic acid (0.05 mM), NaH_2PO_4 (2.2 mM), CaCl_2 (2.2 mM) and the pH was adjusted on 4.5 using NaOH 50%. The solutions were changed every day to avoid aggregation of demineralization products and pH change. After 96 hours, first, every specimen surface was washed thoroughly using a syringe containing artificial saliva and then all the specimens were kept in containers containing artificial saliva and were used again to measure DIAGNOdent value. (In this study, artificial saliva was prepared with formula NaCl (4.2mM), Na_3PO_4 (3.9mM), H_2SO_4 (0.5mM), MgCl_2 (0.08mM), CaCl_2 (1.1mM), KCl (17.9mM) and NaHCO_3 (3.2mM)).

For the G1group, Fluoride varnish (Sodium Fluoride, Sultan, USA) was placed on the specimens (and air dried for 30 sec) according to

manufacturer instruction. Then, the specimens were immersed in artificial saliva and removed after 1 hour and the Fluoride varnish on their surfaces was cleaned gently from the enamel using scalpel blade (surgical blade, no 15, Pakistan) and the surface of every specimen was washed using a syringe containing artificial saliva. For the G2 group, the surfaces of the specimens were treated by CPP-ACP (GC tooth Mousse, Japan) for 3 min according to the manufacturer instruction. Then, the specimens were immersed in artificial saliva and removed after 1 hour and their surfaces were washed using a syringe containing artificial saliva. For the G3 group, all the stages were the same as the G2 group with the difference in using Remin Pro for surface treatment.

In the G4 group, the specimens were treated by Fluoride varnish for 1 month, daily 8 hours, so that after drying and impregnating of specimens surfaces with Fluoride varnish, the premounted samples were reserved in special closed containers. 1/3 of each container were filled with artificial saliva in order to prevent drying the varnish. Samples were kept 8 hours a day in these containers and the treated surfaces were out of saliva.

During the period of treatment, treated surfaces became moist twice a day by one drop of artificial saliva. Then, same as the first group, the surfaces were cleaned from fluoride Varnish, they were washed and the specimens were immersed in artificial saliva for 16 hours. This was done every day for 1 month. For the G5 group, the specimens were treated for 1 month, 8 hours a day, with CPP-ACP paste. All treatment regimens were the same as the G4 group except for that cotton swab was used to remove CPP-ACP from the surface of the specimens. For the G6 group, all the stages were the same as the G5 group with the difference that Remin-Pro was used for surface treatment. After surface treatment for the six above-mentioned groups, DIAGNOdent value was re-measured and

recorded for all the specimens.

At this stage G4, G5, and G6 were exposed to the pH-cycling regimen in order to mimic the natural cycle in the mouth.

pH-cycling includes demineralization (3hours) and remineralization (21 hours) for 5 consecutive days. After pH-cycling, DIAGNOdent values of the specimens were measured again.

Table 1- DIAGNOdent @ interpretation

Moment value	Inference
0-7	Healthy tooth substance
8-15	Beginning demineralization
>16	String demineralization

One way ANOVA, repeated measures ANOVA and least significant difference (LSD) test were used to statistical analysis.

Results:

One way ANOVA test indicated that the six groups had no significant differences in terms of mean baseline DIAGNOdent values ($p=0.67$).

Repeated measures ANOVA indicated that the mean of DIAGNOdent values in each group was significantly different between three stage ($p<0.001$).

In data analysis the power of test was 80%.

After the specimens were placed in demineralizing solution, the mean of DIAGNOdent value increased significantly for all groups, however, the difference between the six groups was not significant ($p=0.5$).

After the treatment, the mean of DIAGNOdent values significantly decreased in groups G4, G5 and G6 ($p<0.001$), however, the decrease was the same for all groups ($p=0.83$) (Figures 1, 2).

From study baseline to re-demineralization stage, DIAGNOdent decrease of values were significantly higher in Remin-Pro and CPP-ACP groups than Fluoride varnish group ($p<0.001$), however CPP-ACP and Remin-Pro groups were not significantly different (Figure 3).

The test also indicated that the mean of DIAGNOdent value, from study baseline to remineralization stage, was significantly higher in Remin-Pro and CPP-ACP groups than

Fluoride varnish group ($p=0.001$), however, CPP-ACP and Remin-Pro groups were not significantly different ($p=0.3$).

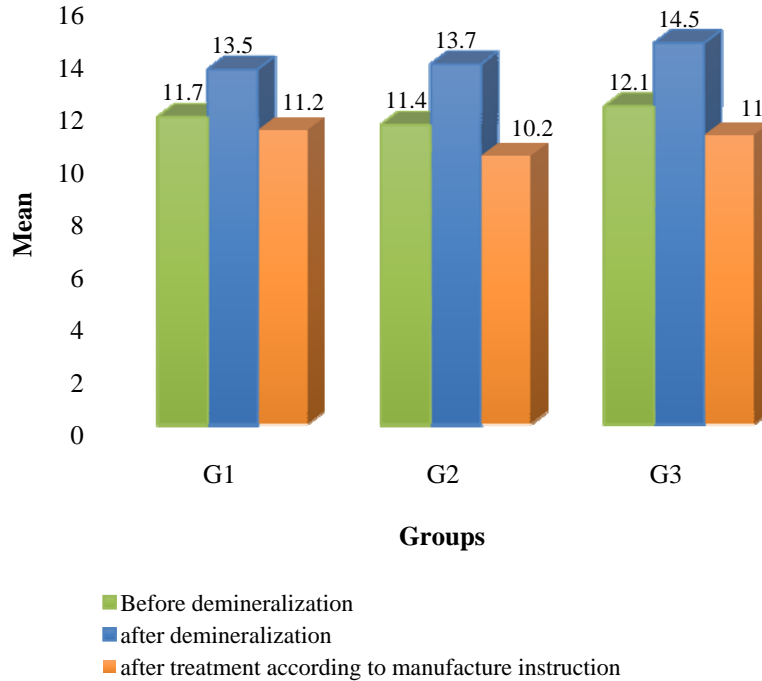


Diagram 1- The mean DIAGNOdent values

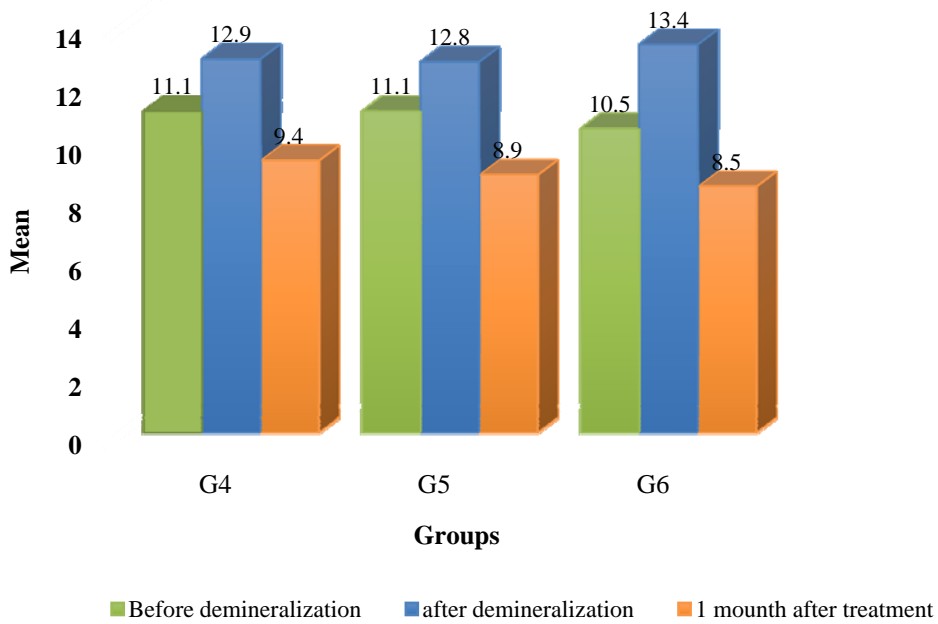


Diagram 2- The mean DIAGNOdent values

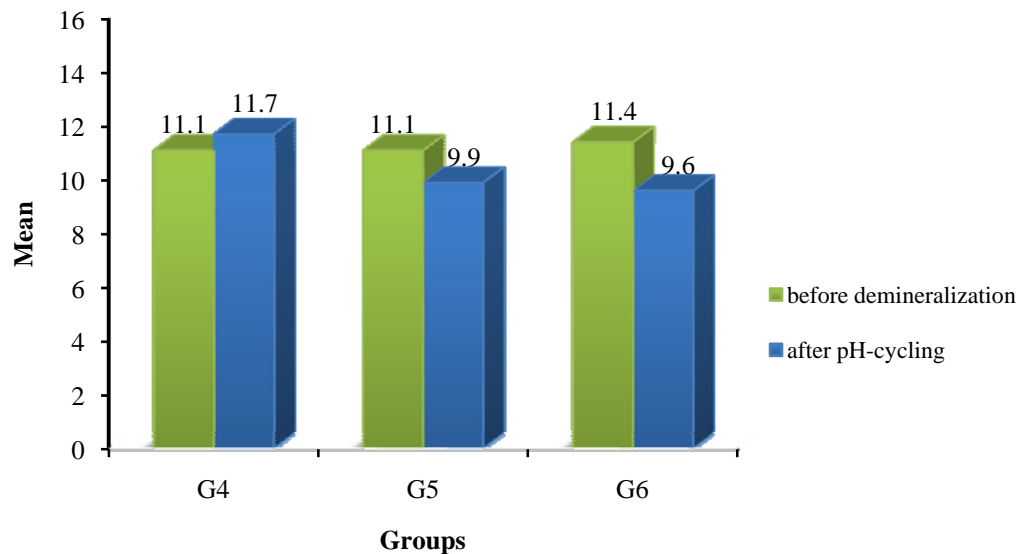


Diagram 3- Mean resistance to demineralization after treatment

Discussion:

Early caries diagnosis in initial stage is crucial, when the preventive agents are effective to stop caries.

Some non-invasive diagnostic methods include quantitative light fluorescence (QLF), trans-illumination fiber optic, coherent optical tomography, laser fluorescence (DIAGNOdent) and electronic microscope. Using the machines DIAGNOdent, based on laser fluorescence and measurement of fluorescent rate of tooth structures, promises a bright future to offer a non-invasive method with reliable and repeatable results (14).

In the present study, DIAGNOdent is used as a non-invasive method of evaluation of tooth surface changes after demineralization and remineralization by preventive materials including Fluoride varnish, CPP-ACP and Remin-Pro. In this non-invasive method, laser fluorescence with wave length 650nm was used. Organic and non-organic material in dental

surfaces absorb laser light and emit fluorescence light in infrared range. Presence of demineralized areas increase fluorescent rate and the operator is informed by hearing some noise.

One of the objectives of prevention is caries stop at early stages and remineralization of dental surfaces. If incipient caries and white enamel lesions can be remineralized by preventive material, cavity development is reduced and more tooth structure could be preserved. In fact, early enamel lesions have remineralization potential with more acid resistance surfaces especially at treatment regimen times (15).

Changing the pattern of caries development not only is related to the type of preventive method but also it depends on the applied strategy to recognize at early stages. One of these strategies is using non-invasive techniques such as laser fluorescence and CPP-ACP paste application.

Fluoride ion presence around tooth surfaces causes fluorapatite to deposit from calcium and phosphate in saliva, increasing pH, new larger crystals containing fluoride and resistance to

acid (fluorohydroxyapatite) form. Forming such crystals causes increase in remineralization, a strong layer form and resistance of tooth structure to demineralization increase (2).

Casein phosphate component in CPP-ACP causes calcium and phosphate fixation and forms nano-complexes of calcium phosphate on the tooth surface which acts as a source of mineral in remineralization and mineral deposition on the tooth surface. In fact, insoluble calcium phosphate becomes soluble in presence of casein phosphopeptide and forms amorphous calcium phosphate which is localized on the tooth surface and is the source of calcium and phosphate ions storage. Amorphous calcium phosphate transfers calcium and phosphate ions to the depth of lesion through the porous layer on the white lesion; diffusing the ions in the lesion, enamel crystals are remineralized and the material is also capable of fast turning to hydroxyapatite in oral environment and will deposit on the surface of the tooth(7, 16-18).

Remin-Pro has Fluoride, Hydroxyapatite and Xylitol in its combination. Fluoride and hydroxyapatite in this material reinforce remineralization and strengthen enamel surface, as well as help acid neutralizing and balance the oral flora. As this material significantly increased remineralization (even compared to Fluoride varnish), it is recommended for conservative treatments, after whitening and during orthodontic treatments. The manufacturer claims that this material prevents dental sensitivity by creating a protective layer on the tooth, and considerably smoothen the tooth surface, therefore, prevents adhesion of bacterial plaques.

This can be attributed to fine particles of amorphous calcium phosphate (nano-complexes) in CPP-ACP paste, as its calcium and phosphate ions easily diffuse in the porous lesion and penetrate to the depth of demineralized lesion due to its fine size (nano) and re-form apatite crystals even in deeper parts; this certainly

depends on long-term treatment and enough time to be exposed to this material. As well as fluoride ion causes an increase in the resistance of tooth surface to demineralization by deposition of fluorohydroxyapatite. Despite its advantages, resulting remineralization is a self-limiting phenomenon that inhibits penetration of calcium and phosphate ions to the lesion depth (2, 19). The above-mentioned issues can justify more the effect of CPP-ACP than Fluoride varnish on increase of remineralization.

In addition, Fluoride ion is able to remineralize demineralized enamel lesions in presence of sufficient amount of calcium and phosphate ions; in fact, sufficient amount of these ions is a restricting factor for their effectiveness. Actually, for every two Fluoride ions, 10 calcium ions and 6 phosphate ions are required to form a cell unit of Fluorapatite (20). Insufficient calcium and phosphate ions when exposing tooth surface to Fluoride varnish might be another reason for lower effect of Fluoride varnish on enamel remineralization.

Jayarajan *et al.* reported that CPP-ACP and CPP-ACPF are excellent delivery vehicles available in a slow-release amorphous form to localize calcium, phosphate, and fluoride at the tooth surface (14).

The mean of DIAGNOdent value for G5 and G6 after demineralization of treated enamel and after pH-cycling is significantly lower than the value at baseline; however, Fluoride varnish is almost similar to the baseline time. It can be attributed to cumulative effect of mineral deposition (Apatite, phosphate and calcium crystals) through long-term treatment periods with all three materials and consequently more resistant treated surfaces than healthy enamel. In other articles, creation of a more resistant layer than healthy enamel when treating with Fluoride and CPP-ACP were also addressed (2-11).

Hegde and Moany (2012) concluded that the CPP-ACP significantly remineralizes synthetic enamel subsurface lesions, and the

remineralization rate increases with increasing duration of treatment with CPP-ACP (21).

Srinivasan *et al.* (2010) concluded that the CPP-ACP paste alone and also associated with fluoride 900 ppm is capable of softened enamel remineralization, and the potential was greater in the group where CPP-ACP and fluoride were used together (22). Rahiotis and Vougiouklakis (2007) concluded that the presence of CPP-ACP on dentin surface can reduce demineralization and increase remineralization (7). The results of these studies are consistent with the results of our study

In the studies of Lata *et al.* (2010), Kim *et al.* (2013) and Pulido *et al.* (2008), the effect of fluoride (either in varnish or toothpaste form containing fluoride) on increased remineralization was mentioned equal to or greater than CPP-ACP. This difference may be due to either the short periods of treatment and

lack of sufficient time for greater penetration of material to the depth of lesions (especially in the case of CPP-ACP) or using distilled water instead of artificial saliva (2, 23, 24).

Conclusion:

Fluoride varnish, CPP-ACP and Remin Pro cause remineralization of early enamel lesions in long-term frequent use. One time application of Fluoride varnish, CPP-ACP and Remin Pro according to manufacturer is not effective on remineralization of caries lesions. According to the results of the present study, the specimens treated with CPP-ACP and Remin-Pro are significantly more resistant to remineralization than Fluoride varnish.

Conflict of Interest: “None Declared”

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